Contents

Organizing Committee

Chair: **Angela Kent**
Department of Natural Resources and Environmental Sciences
University of Illinois Urbana-Champaign

Chair: **Anthony Yanarell**
Department of Natural Resources and Environmental Sciences
University of Illinois Urbana-Champaign

Shannon Sirk
Department of Bioengineering
Director, Microbial Systems Initiative
University of Illinois Urbana-Champaign

Brett Lowman
Department of Animal Sciences
University of Illinois Urbana-Champaign

Sara Ressing
Microbial Systems Initiative
University of Illinois Urbana-Champaign

Meghan Blaszynski
Graduate Student, Plant Biology
University of Illinois Urbana-Champaign

Hannah Murray
Graduate Student, Program in Ecology Evolution and Conservation
University of Illinois Urbana-Champaign

Fahren Zackery
Graduate Student, Program in Ecology Evolution and Conservation
University of Illinois Urbana-Champaign

Incoming Host Team
Laramy Enders
Department of Entomology
Purdue University

Stephen Lindemann
Department of Food Science
Purdue University

Symposium Academic Sponsors

Cancer Center at Illinois

Carl R. Woese Institute for Genomic Biology

College of Agricultural, Consumer & Environmental Sciences

College of Veterinary Medicine

Department of Agricultural & Biological Engineering

Department of Bioengineering

Department of Crop Sciences

Department of Food Science & Human Nutrition

Department of Microbiology

Department of Natural Resources & Environmental Sciences

Genomics and Eco-evolution of Multi-scale Symbioses (GEMS)

Personalized Nutrition Initiative

Roy J. Carver Biotechnology Center

School of Molecular & Cellular Biology

Olga G. Nalbandov Lecture Fund

Symposium Corporate Sponsors

BiomEdit is an innovation company that discovers, designs, and develops novel microbiome-derived products to address unmet needs in animal health.

Earnest Agriculture is an ag-tech startup that develops microbial combinations that are applied as a seed coating to enhance crop resilience and increase yields. Our goal is to help farmers maintain sustainable margins by reducing their overall input costs through the use of microbe-based solutions. We have grown our footprint to over 10,000 acres of US farmland and have conducted field trials across 14 states at 50 locations. We are also supported by leading organizations including AgLaunch, Dairy Farmers of America, IndieBio, and the University of Illinois.

Frontiers is one of the world's largest and most impactful research publishers, dedicated to making peer-reviewed, quality-certified science openly accessible. With over three million researchers across 222 community-led journals covering approximately 1,700 academic disciplines, we provide researchers with a trusted, cutting-edge, AI-powered open science platform to rigorously review their findings and maximize the dissemination of their discoveries. As an open access pioneer, we actively drive the global transition to open science, working with researchers, universities, educators, policymakers, and businesses.

Metabolon is a global leader in metabolomics, providing advanced biochemical profiling and multiomics solutions that help researchers better understand health, disease, and biological systems. Through its industry-leading platforms and extensive metabolite library, Metabolon delivers high-quality data and actionable insights that accelerate biomarker discovery, precision medicine, and life sciences research. By measuring the metabolites produced and modified by microbial communities, Metabolon reveals the functional output of the microbiome, providing critical insight beyond sequencing alone and connecting microbial activity to host biology. This approach empowers researchers to uncover mechanisms, identify biomarkers, and translate microbiome discoveries into real-world impact.

Trainees Social Activities

Trainee Social
Monday, May 11 | 6:30 – 8:30 PM
Riggs Beer Company, 1901 S High Cross Rd, Urbana, IL 61802

Join fellow trainees for an informal evening social at Riggs Beer Company. Food will be provided by Black Dog Smoke & Ale House. Transportation and parking information will be shared during the symposium.

Early Morning Walk to the Arboretum
Tuesday, May 12 | 7:30 – 8:15 AM
University of Illinois Arboretum, 1800 S Lincoln Ave, Urbana, IL 61801

Start the day with a guided walk to the University of Illinois Arboretum. Participants will explore one of the University of Illinois campus's major green spaces while learning about the collections, landscapes, and ecological features of the arboretum. Participants should be prepared for outdoor walking. Departs from the I-Hotel Lobby at 7:15 AM.

Community Social Event
Tuesday, May 12 | 6:30 – 7:30 PM
I Hotel & Illinois Conference Center, 1900 S 1st St, Champaign, IL 61820

An evening community-building event featuring trivia, crafts, board games, and informal networking spaces for attendees across disciplines and career stages. Pizza and snacks will be provided.

Career Panel

Wednesday, May 13 | 12:30 – 1:15 PM
I Hotel & Illinois Conference Center, 1900 S 1st St, Champaign, IL 61820

A panel discussion featuring researchers and professionals from academia, national laboratories, industry, and interdisciplinary careers sharing insights on career paths, professional development, and navigating opportunities in the microbiome sciences.

Chris Fields
Director, High-Performance Biological Computing, Biotechnology Center
University of Illinois Urbana-Champaign

Ran Mei
Assistant Professor, Department of Civil and Environmental Engineering
University of Illinois Urbana-Champaign

Caroline Oldstone-Jackson
Postdoctoral Researcher
University of Illinois Urbana-Champaign

Justin Podowski
Assistant Computational Biologist
Argonne National Laboratory

Gabe Price
Co-founder, Earnest Agriculture; Gies College of Business
University of Illinois Urbana-Champaign

Sierra Raglin
Postdoctoral Researcher
University of Illinois Urbana-Champaign

Nídia Sequeira Trovão
Assistant Professor, Department of Pathobiology
University of Illinois Urbana-Champaign

Schedule

All events are located in Heritage Hall at the Illinois Conference Center (1902 S 1st St, Champaign, IL 61820) unless otherwise noted.

Monday, May 11th, 2026	
Time	**Agenda**
12:00 – 12:30 pm	Registration and light snacks
12:30 – 12:45 pm	Welcome Remarks Angela Kent & Anthony Yannarell, University of Illinois Urbana-Champaign
Session 1 12:45 – 2:00 pm	**Microbial Metabolites in Health and Disease** Chair: Sierra Raglin, University of Illinois Urbana-Champaign
12:46 – 1:00 pm	*Ultra-marathon running reshapes gut microbial aromatic amino acid metabolism and disrupts barrier function* **Casey Lim**, University of Illinois Urbana-Champaign
1:01 – 1:15 pm	*Indole toxicity on removal of uremic toxin p-cresol, in-vitro study of Thauera aminoaromatica S2* **Pei-Hsin Wang**, University of Washington
1:16 – 1:30 pm	*Dietary fiber fermentability and viscosity influence gut microbial metabolites in chronic kidney disease: A systematic review and meta-analysis of animal and clinical trials* **Seyedeh Nooshan Mirmohammadali**, Purdue University
1:31 – 1:45 pm	*Establishing in vivo and in vitro approaches to investigate urobiome metabolism of environmental chemicals and bladder cancer development* **Audra Crouch**, Ohio State University
1:46 – 2:00 pm	*Short-term mixed-fiber supplementation associates with selective mucosal microbial shifts and alterations in mucosal metabolism* **David Alvarado**, University of Illinois Urbana-Champaign
2:00 – 2:30 pm	Coffee break
Session 2 2:30 – 4:00 pm	**Farm to Food – Microbes Across the Food Supply** Keynote: Melha Mellata, Iowa State University Chair: Brett Loman, University of Illinois at Urbana-Champaign
2:31 – 2:45 pm	*Effects of high soybean meal inclusion on the gut microbiome of finishing pigs* **Angel Martinez**, South Dakota State University
2:46 – 3:00 pm	*Acute azoxystrobin exposure induces gut microbiome disruption and hepatic transcriptional changes in zebrafish* **Luoyan Duan**, University of Illinois Urbana-Champaign
Keynote 3:01 – 4:00 pm	***The Avian Segmented Filamentous Bacteria: A master of gut immunity and microbiome development in early life*** **Melha Mellata**, Iowa State University

4:00 – 5:30 pm	**Poster Session 1** with light snacks Even number posters presenting
6:30 – 8:30 pm	Trainee Social Event Riggs Brewery, 1901 S High Cross Rd, Urbana, IL 61802

Tuesday, May 12th, 2026	
Time	**Agenda**
7:30 – 8:15 am	Morning walk - Meet at 7:20 in the iHotel Lobby University of Illinois Arboretum, 1800 S Lincoln Ave, Urbana, IL 61801
8:00 – 8:45 am	Light breakfast and coffee service
8:45 am	Welcome
Session 3 9:00 – 10:30 am	**Microbes and Our Water** Keynote: Erica Hartmann, Northwestern University Chair: Wei Qin, University of Illinois at Urbana-Champaign
9:01 – 9:15 am	*Impact of hybrid capture enrichment and sample preparation methods on ARG detection in wastewater* **Christopher Owen**, University of Illinois Chicago
9:16 – 9:30 am	*Combinatorial characterization of drinking water biofilm communities reveals both synergies and negative interactions affecting biomass and chloramine tolerance* **Jessica Li**, University of Michigan
Keynote 9:31 – 10:30 am	***Ecology and evolution of antimicrobial resistance: the environmental perspective*** **Erica Hartmann**, Northwestern University
10:30 – 11:00 am	Coffee break
Session 4 11:00 – 12:30 pm	**Microbial Drivers of Nitrogen Cycling** Keynote: Lydia Zeglin Chair: Angela Kent, University of Illinois at Urbana-Champaign
11:01 – 11:15 am	*Fertilization-conditioned soil microbiomes shape cultivar-dependent assembly of soybean nodule microbiomes* **Danyang Duan**, University of Illinois at Urbana-Champaign
11:16 – 11:30 am	*A resource allocation framework for denitrification* **Dominic Cipti**, The Ohio State University
Keynote 11:31 – 12:30 pm	***Bison and cattle grazing affect the diversity and function of soil nitrogen cycling microbes in native tallgrass prairie*** **Lydia Zeglin**, Kansas State University
12:30 – 2:00 pm	Lunch (box lunches provided) + Sponsored talks Chaired by Angela Kent & Anthony Yannarell
12:50 – 1:30 pm	Roy J. Carver Biotechnology Center sponsored session

1:30 – 1:45 pm	Metabolon talk: Multiomics microbiome datasets – Josh Simpson
Session 5 2:00 – 3:30 pm	**Bugs in Bugs** Keynote: Cameron Currie Chair: Matt Doremus, University of Illinois at Urbana-Champaign
2:01 – 2:15 pm	*Genome-based identification of a novel 'Candidatus Phytoplasma' species from environmental insect samples in China* **Valeria Trivellone**, University of Illinois Urbana-Champaign
2:16 – 2:30 pm	*Effects of Oscillating Temperatures on Reproductive Manipulating Endosymbionts in Spider Host* **Emily Cook**, University of Illinois at Urbana-Champaign
Keynote 2:31 – 3:30 pm	***Ancient microbiome husbandry in fungus-farming ants*** **Cameron Currie**, McMaster University
4:00 – 5:30 pm	**Poster Session 2** with light snacks Odd number posters presenting
6:30 – 7:30 pm	Trainee Social Activity Games and crafts in Heritage Hall

Wednesday, May 13th, 2026	
Time	***Agenda***
8:00 – 8:45 am	Light breakfast and coffee service
8:00 – 8:45 am	Midwest Multidisciplinary Microbiome Symposia Board Meeting (closed session – board members only) Houlihan's, 1902 S 1st St, Champaign, IL 61820
8:45 am	Welcome
Session 6 9:00 – 10:30 am	**Microbial Ecology** Keynote: Jay Lennon, Indiana University Bloomington Chair: Anthony Yannarell, University of Illinois Urbana-Champaign
9:01 – 9:15 am	*Untargeted metabolomics reveals metabolic differences between symbiotic and aposymbiotic phenotypes of a facultatively symbiotic temperate coral (Astrangia poculata)* **Erik Andersson**, University of Illinois Chicago
9:16 – 9:30 am	*SIPdb: a stable isotope probing database for extending ecological interpretation to amplicon datasets.* **Alex Batista Trentin**, Purdue University
Keynote 9:31 – 10:30 am	***Microbiomes and climate action: Connecting society initiatives to global change*** **Jay Lennon**, Indiana University Bloomington
10:30 – 11:00 am	Coffee break

Session 7 11:00 – 12:00 pm	**Dietary Fiber, Health, and Resilience** Chair: Shannon Sirk, University of Illinois at Urbana-Champaign
11:01 – 11:15 am	*Whole food fibers for support of key gut bacteria for human health* **Lauren Sofia Yepes**, Purdue University.
11:16 – 11:30 am	*A dual-color intestinal co-culture platform to evaluate host responses to inulin-derived microbial metabolites* **Yuxin Wang**, Purdue University
11:31 – 11:45 am	*Influence of diet on stress-induced HPA axis activation* **Jordan Rindels**, University of Illinois at Urbana-Champaign
11:46 – 12:00 pm	*Gut microbiome and behavior effects on Travelers' Diarrhea occurrence in a military population* **Zachary Liechty**, Air Force Research Laboratory
12:00 – 1:30 pm	Lunch – Box Lunches Provided
12:30 – 1:15 pm	Trainee Career Panel
Session 8 1:30 – 2:15 pm	**Discovering Microbial Functions** Chair: Patrick Bradley, Ohio State University
1:31 – 1:45 pm	*Highly efficient preparation of large functional metagenomic libraries and their application to capture novel antimicrobial resistance genes* **Terence Crofts**, University of Illinois at Urbana-Champaign
1:46 – 2:00 pm	*Assessing the reproducibility of whole-microbial community evolution of AMR: unraveling the contributions of vertical and horizontal gene transfer* **Lisa Stabryla**, University of Illinois at Chicago
2:01 – 2:15 pm	*CheckAMG and PhAuxDB enable ecosystem-scale discovery and pangenomic classification of auxiliary viral genes* **James Kosmopoulos**, University of Wisconsin, Madison
2:16 – 2:30 pm	*Uncovering biosynthetic gene clusters in phage and giant virus genomes* **Neha Kashyap**, University of Wisconsin, Madison
2:30 – 2:50 pm	Closing ceremony and awards

Keynotes

The Avian Segmented Filamentous Bacteria: A master of gut immunity and microbiome development in early life
Melha Mellata, Iowa State University
Monday, May 11 | 3:01 – 4:00 PM

Dr. Melha Mellata is an associate professor of molecular microbiology in the Department of Food Science and Human Nutrition, and the Director of the interdepartmental Microbiology Graduate program at Iowa State University. Her research interests lie in the areas of the pathogenesis of bacterial diseases, prophylactic development, and gut microbiota. The long-term goal of the research program is to develop effective intervention strategies against bacterial infections and antibiotic resistance. Her research will benefit both human health and agricultural animal production and will enhance food safety by reducing the transmission of bacteria through the animal-food production pipeline.

Ecology and evolution of antimicrobial resistance: The environmental perspective
Erica Hartmann, Northwestern University
Tuesday, May 12 | 9:31 – 10:30 AM

Dr. Erica Marie Hartmann began her research career at the Johns Hopkins Bloomberg School of Public Health, where she worked as on mass spectrometry-based methods for detecting microbial enzymes necessary for bioremediation. From Hopkins, she moved to Arizona State University where she was the first graduate of the interdisciplinary Biological Design PhD program. Following her graduation, she was awarded a Fulbright to study microbes that degrade the toxic, carcinogenic pollutants known as dioxins in France at the Commission for Atomic Energy. She began leading studies on the effects of antimicrobial chemicals on the microbes found in indoor dust at the Biology and the Built Environment Center at the University of Oregon and is currently continuing that work as an associate professor at Northwestern.

Bison and cattle grazing affect the diversity and function of soil nitrogen cycling microbes in native tallgrass prairie
Lydia Zeglin, Kansas State University
Tuesday, May 12 | 11:31 AM – 12:30 PM

Dr. Lydia Zeglin is an Associate Professor in the Division of Biology at Kansas State University (KSU); she is a microbial and ecosystem ecologist whose research links microbial ecology, microbial diversity, and nutrient cycling processes in soils and streams. The Zeglin Lab's main goal is to build knowledge on the maintenance and sustainability of managed and 'natural' ecosystem services, within the context of disturbance and global change, through the integration of perspectives at microbial and ecosystem scales. Dr. Zeglin's research is a multi-site and interdisciplinary collaborative endeavor that advances the understanding of how microbial biodiversity supports ecosystem processes in diverse freshwater and terrestrial ecosystems. For example, ongoing work illuminates aspects of soil microbial diversity underlying key carbon and nitrogen cycling processes, within the contexts of ecosystem recovery from chronic nitrogen fertilization and drought, and supports many advances in grassland ecology and biogeochemistry. The Zeglin Lab studies microbial roles in soil carbon storage and nitrogen efficiency in grasslands globally, with particular interest in identifying microbial metabolisms and keystone relationships that define this valuable biodiversity. Dr. Zeglin was the recipient of an NSF Early Career Award, was named a University Outstanding Scholar at KSU, and is a co-PI of the Konza Prairie Long-Term Ecological Research (LTER) project and a co-Investigator on the McMurdo Dry Valleys LTER project.

Ancient microbiome husbandry in fungus-farming ants
Cameron Currie, McMaster University
Tuesday, May 12 | 2:31 – 3:30 PM

Dr. Cameron Currie is the Mr. Jarislowsky Chair in Pandemic Research and Prevention in the Department of Biochemistry and Biomedical Sciences at McMaster University. Dr. Currie's research pioneered the study of multipartite symbioses and defensive microbiomes, with seminal work on the microbiome of fungus-growing ants revealing ancient coevolutionary dynamics between hosts, mutualists, and pathogens. This work, which began during their doctoral studies at the University of Toronto, has fundamentally shaped our understanding of symbiotic complexity in nature. Dr. Currie has published over 190

papers cited more than 22,800 times (h-index: 86), with publications in Nature, Science, PNAS, and other top-tier journals. Their highly interdisciplinary approach integrates microbiology, evolutionary biology, genetics, and chemistry to understand host-microbe interactions across systems. Dr. Currie has received numerous honors, including the 2001 NSERC Doctoral Prize, the 2008 Presidential Early Career Award in Science and Engineering (PECASE) from President Obama, and election as a Fellow of the American Academy of Microbiology in 2013. Prior to joining McMaster in 2022, they held faculty positions at the University of Kansas and the University of Wisconsin-Madison, where they served as the Ira L. Baldwin Professor of Bacteriology.

Microbiomes and climate action: Connecting society initiatives to global change

Jay Lennon, Indiana University Bloomington

Wednesday, May 13 | 9:30 – 10:30 AM

Dr. Jay Lennon is a Distinguished Professor in the Department of Biology at Indiana University and former Chair of the Evolution, Ecology, and Behavior (EEB) Section. He teaches a computationally focused graduate course in Quantitative Biodiversity and an undergraduate course on Microbiomes: Host and Environmental Health, and was an instructor for the Microbial Diversity course at the Marine Biological Laboratory (MBL) in Woods Hole. Previously, Lennon was an Assistant and Associate Professor in the Department of Microbiology & Molecular Genetics and the W.K. Kellogg Biological Station (KBS) at Michigan State University. He completed postdoctoral training at Brown University and earned a Ph.D. at Dartmouth College, a Master's degree at the University of Kansas, and a Bachelor's degree at the SUNY College of Environmental Science and Forestry (ESF) at Syracuse University. Lennon's research team is motivated by the ecological and evolutionary processes that generate and maintain microbial biodiversity. In turn, they investigate the implications of diversity for the stability and functioning of ecosystems using molecular biology, mathematical modeling, data synthesis, laboratory experiments, and field work in a wide range of habitats. His group has shed light on the role of functional traits for predicting community dynamics. They have documented the importance of dormancy, whereby individuals can enter a reversible state of reduced metabolic activity. Recent work has shown how the

resulting seed banks lead to the emergence of complex phenomena. His group also integrates microbial life forms with other taxa across the tree of life to test macroecological theory. Such efforts have unveiled scaling laws which predict Earth is home to one trillion (1012) species. Lennon is actively engaged in scientific leadership. He has served on the Governing Boards of both the American Academy of Microbiology (AAM) and the Ecological Society of America (ESA). Within the AAM, he chairs the Climate Change & Microbes Scientific Portfolio, where he fosters partnerships across academia, non-profits, foundations, and the private sector to advance science and policy. He is also a founding member of a global coalition that brings together microbiological societies to identify and implement climate change solutions. Most recently, Lennon was appointed Chair of the Scientific Unit on Applied and Environmental Microbiology at the American Society for Microbiology (ASM), a new initiative designed to address global sustainability challenges through the scientific foundations of microbiology. Lennon has published more than 150 papers with competitive awards from the National Science Foundation (NSF), the National Institutes of Health (NIH), the National Aeronautics and Space Administration (NASA), the Department of Defense, (DoD), United States Department of Agriculture (USDA), and the United States Geological Survey (UGSG). Among other recognitions, Lennon has been elected to the American Association for the Advancement of Science (AAAS), the American Academy of Microbiology (AAM), and the Ecological Society of America (ESA). He was named an ASM Distinguished Lecturer, is a recipient of the Humboldt Prize from the Alexander von Humboldt Foundation, and was selected as a Kavli Fellow by the National Academy of Sciences

Abstracts (Oral Presentations)

Ultra-Marathon Running Reshapes Gut Microbial Aromatic Amino Acid Metabolism and Disrupts Barrier Function

Casey KY. Lim[1], Mikaela C. Kasperek[2], James R. Bagley[3], Gregory J. Grosicki[4], Jacob M. Allen[1,2]

1. Department of Health and Kinesiology, University of Illinois at Urbana-Champaign, 906 S. Goodwin Ave, Urbana, IL 61801|
2. Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, 906 S. Goodwin Ave, Urbana, IL 61801

3. Department of Kinesiology, San Francisco State University, 1600 Holloway Avenue, GYM 101, San Francisco, CA 941324
4. Department of Performance Science, WHOOP, Inc, 1 Kenmore Square, Boston, MA 02215

Background: The gut microbiome plays a central role in the metabolism of aromatic amino acids (ArAA), producing bioactive metabolites through both oxidative and reductive pathways. Microbial metabolism is dynamic and responsive to physiological stress that disrupts the gut microbiome and epithelial integrity. Whether this is associated with shifts in ArAA metabolism toward oxidative or reductive pathways that relate to barrier function remains unknown.

Purpose: To characterize pre- to post- ultra-endurance race changes in gut microbial composition, ArAA-derived metabolites, and signs of barrier disruption in participants of the 2023 Western States Endurance Run (100 miles).

Methods: Runners (n=37) provided pre- and post-race stool (≤72 h) and serum (<1 hr). Gut microbiome composition was analyzed using 16S rRNA sequencing, including measures of α- and β-diversity and relative abundance of *Streptococcus*. Circulating and fecal ArAA metabolites were quantified via targeted LC-MS and grouped as oxidative (aryl-acetates) or reductive (aryl-lactates), reflecting divergent microbial redox pathways. Intestinal epithelial injury and barrier disruption were assessed by plasma intestinal fatty acid–binding protein (I-FABP; enterocyte damage marker) and lipopolysaccharide-binding protein (LPS-BP; marker of systemic endotoxin exposure).

Results: Microbial α- and β-diversity were unchanged after ultra-marathon; however, *Streptococcus* abundance increased ($p = 0.0083$). Circulating oxidative aryl-acetates increased ($p = 0.0057$) while reductive aryl-lactates decreased ($p = 0.0039$), supporting a shift toward oxidative microbial metabolism. Fecal aryl-acetates and aryl-lactates were not significantly altered ($p = 0.1926$, $p = 0.0721$), although the overall directional pattern was consistent. I-FABP and LPS-BP increased post-race ($p = 0.0245$, $p = 0.0001$). There was a positive correlation between I-FABP and race time was $\rho = 0.49$ ($p = 0.078$), indicating a relationship between extreme exercise performance and gut barrier integrity.

Conclusion: Acute ultra-marathon participation associates with shifts in gut microbial ArAA metabolism toward oxidative pathways without broad changes in overall microbial diversity, supporting functional changes in microbial amino acid metabolism under extreme physiological stress.

Indole toxicity on removal of uremic toxin p-cresol, in-vitro study of Thauera aminoaromatica

Pei-Hsin Wang[1,‡], Prakit Saingam[1,‡], Rosita Rasyid[1], Bruce J.Godfrey[1], Jonathan Himmelfarb[2], Mari Karoliina Henriikka Winkler[1]

‡PS and PW contributed equally to this work as co-first authors
[1] Departmental Affiliation: Department of Civil and environmental engineering, University of Washington
[2] Icahn School of Medicine

Protein-bound uremic toxins such as indoxyl sulfate and p-cresyl sulfate are key contributors to chronic kidney disease (CKD) complications and are poorly removed by dialysis due to strong albumin binding. Targeting their gut-derived microbial precursors represents a promising strategy to reduce systemic toxin load. *Thauera aminoaromatica* S2 is known to anaerobically degrade p-cresol, but its response to indole and its performance as orally administered microbial therapy remain insufficiently characterized. Here, we investigated the activity and concentrations of indole and p-cresol in exposure to planktonic and hydrogel encapsulated *Thauera aminoaromatica* S2. *Thauera aminoaromatica* S2 exposed to 2 mM p-cresol and 0.25 mM indole exhibited enhanced growth. In contrast, encapsulated cells exhibited slower p-cresol degradation when co-exposed to indole, suggesting that hydrogel matrices may restrict indole outward diffusion and increase local indole concentrations, thereby intensifying cellular stress. The incorporation of activated carbon into the hydrogel matrix restored p-cresol removal despite indole exposure, likely through localized indole sequestration. These results highlight the potential of combining encapsulation materials with adsorptive additives to stabilize microbial function and support the development of microbial therapies aimed at mitigating uremic toxin precursors in CKD

Dietary fiber fermentability and viscosity influence gut microbial metabolites in chronic kidney disease: A systematic review and meta-Analysis of animal and clinical trials

Seyedeh Nooshan Mirmohammadali[1], Claudia Carrillo[2] , Jason B. Reed[3], Brandon Kistler[1], Hannah E Wilson[4], Bruce Hamaker[5], Sharon Moe[6] , Annabel Biruete[1,6*]

1. Department of Nutrition Science, Purdue University, West Lafayette, IN, USA
2. Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, United States
3. Libraries and School of Information Studies, Purdue University, West Lafayette, IN, USA
4. Indiana University School of Medicine, Indianapolis, IN, United States
5. Whistler Center for Carbohydrate Research and Department of Food Science, Purdue University, 745 Agriculture Mall Drive, West Lafayette, IN, USA.
6. Division of Nephrology and Hypertension, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

*Corresponding author: Annabel Biruete, abiruete@purdue.edu, +1 (765) 494-0101

Objectives: To systematically evaluate the effects of isolated dietary fiber interventions on microbially-derived metabolites in chronic kidney disease (CKD), and to determine whether fiber fermentability and viscosity modify metabolite responses across both human trials and animal models.

Methods: A systematic review and meta-analysis of randomized controlled trials and animal studies was conducted searching PubMed, Embase, Web of Science, and CENTRAL (search through 02/06/2026). Eligible studies reported ≥1 gut-derived metabolite, including indoxyl sulfate (IS), p-cresyl sulfate (pCS), trimethylamine-N-oxide (TMAO), tryptophan-derived indoles, or short-chain fatty acids (SCFAs). Random-effects models were used for pooled estimates using weighted mean differences (WMD) for human studies and standardized mean differences (SMD) for animal studies, and subgroup analyses evaluated fiber fermentability, viscosity, dose (<16 vs ≥16 g/day equivalent), intervention duration (≤8 vs >8 weeks), and CKD stage. Risk of bias (ROB-2, SYRCLE) and certainty (GRADE) were assessed.

Results: Twenty-eight studies (13 human, 15 animal) were included, comprising 511 participants and 312 animals with CKD. Across models, fiber supplementation, primarily fermentable and non-viscous fibers, reduced IS (human: −0.13 mg/dL; 95% CI: −0.25, −0.01; p = 0.03; animal: −1.99; 95% CI: −3.06, −0.92; p < 0.0001) and pCS (human: −0.23 mg/dL; 95% CI: −0.46, 0.001; p = 0.051; animal: −1.56; 95% CI: −2.08, −1.03; p < 0.0001). SCFAs increased in animal studies, including cecal acetate (2.00, 95% CI: 0.78 to 3.22; p = 0.001) and circulating propionate (1.51, 95% CI: 0.054 to 2.96; p=0.04)). Further, there were no dose-dependent effects (p>0.05), but longer interventions (>8 weeks) tended to lower pCS (−0.26 mg/dL, 95% CI: −0.55 to 0.02; p=0.06). Some heterogeneity and low-to-moderate certainty were observed.

Conclusions: This is the first systematic review and meta-analysis of both human and animal models in CKD examining the effects of isolated dietary fiber on gut-derived uremic toxins. Our results demonstrate that fermentability is a key determinant of metabolic effects, but that there is minimal work exploring viscous fibers. These findings highlight a mechanistic rationale for greater consideration of functional characteristics of fiber in dietary guidance.

Establishing in vivo and in vitro approaches to investigate urobiome metabolism of environmental chemicals and bladder cancer development

Audra L. Crouch, David Jegede, Brandon McKenna, Madison Griffin, Sheryl Justice, Ahmad El Hellani, Vanessa L. Hale

Department of Veterinary Preventive Medicine, The Ohio State University

Interactions between host cells, environmental chemicals, and microbes play a critical role in bladder health and bladder diseases, including bladder cancer (BC).

Polycyclic aromatic hydrocarbons (PAHs) are environmental chemicals linked to bladder cancer that are a byproduct of incomplete combustion and are prevalent in charred foods, automobile exhaust, and cigarette smoke, with diet and tobacco smoke serving as primary exposure routes. Benzo[a]pyrene, a prototypical PAH, can be metabolized into secondary metabolites that induce pro-inflammatory, mutagenic, and carcinogenic responses in host tissues. While human cells are known to metabolize BaP, it remains unclear whether bladder-associated microbes can metabolize BaP into reactive intermediates and contribute to inflammatory, mutagenic, or carcinogenic effects in bladder tissues. Importantly, previous studies have shown compositional differences between the urinary microbiome of healthy individuals and those with BC, suggesting potential microbial contributions to disease pathogenesis. Finally, it remains unknown if urinary microbiomes remain stable or dysbiotic after long-term exposures to BaP. To address these gaps, we combined ***in vivo* and *in vitro* approaches** to investigate microbial contributions to BaP metabolism and its impact on bladder health. *In vivo*, mice (N = 36) were gavaged daily with BaP for four weeks, and fecal and urine samples were collected weekly for targeted metabolomics, 16S rRNA amplicon sequencing, and metagenomic sequencing. In a parallel *in vitro* study, a bladder-associated microbial isolate *Pseudomonas aeruginosa* was **co-cultured in the presence of but sans contact with urothelial cells (HBLAK) in a trans-well system, enabling BaP diffusion between compartments**. HBLAK cells exposed to BaP with or without *Pseudomonas* showed increased cytotoxicity in a dose-dependent manner. Cytotoxicity of HBLAK cells increased from 5.5% with BaP alone to 17% when BaP was combined with *Pseudomonas aeruginosa*. Spent media will be analyzed for BaP and its metabolites via targeted metabolomics. In the *in vivo* mouse study, we anticipate distinct microbial community differences between treatment groups, characterized by an increased abundance of taxa involved in BaP and secondary metabolite metabolism. This methodological framework enables controlled assessment of host-microbe-chemical interactions and PAH metabolism across biological system, providing a foundation for elucidating microbial contributions to bladder cancer development.

Short-term mixed-fiber supplementation associates with selective mucosal microbial shifts and alterations in mucosal metabolism

David A. Alvarado[1], Lydia Okyere[1], Amber O'Connor[2], Siting Chen[2], V Liana Tsikitis[2], Christopher A. Gaulke[1,3,4,5]

[1]Department of Pathobiology, University of Illinois Urbana-Champaign
[2]Division of GI and General Surgery, Oregon Health & Science University
[3]Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign
[4]National Center for Supercomputing Applications, University of Illinois Urbana-

Champaign
[5]Interdisciplinary Health Sciences Institute, University of Illinois Urbana-Champaign

Background*:* Dietary fiber is a key modulator of colorectal health, yet ~95% of Americans do not meet the recommended adequate intake. Over-the-counter fiber supplements are recommended in routine anorectal care to improve stool form and reduce straining. Mixed-fiber formulations combine physicochemical properties that may shape the rectal mucosal environment through coordinated effects on microbiota composition, SCFA handling, and host transcriptional programs. We aimed to characterize multi-compartment changes associated with a pragmatic mixed-fiber add-on.

Methods*:* In a 28-day clinical intervention, participants added 15g/day mixed fibers (7.5g psyllium husk, 8g wheat dextrin) to their habitual diet. Rectal mucosal biopsies and serum were collected at baseline (PRE) and post-intervention (POST). Final analyses included participants whose POST biopsy occurred within 7 days of the final dose (n=37). The microbiota was profiled by 16S rRNA gene sequencing. Targeted and untargeted metabolomics were quantified in tissue and serum by GC-MS and LC-MS. Host mucosal RNA-seq was performed in a paired subset (n=10), with pathway analysis assessed using KEGG gene set enrichment analysis (GSEA).

Results*:* The fiber supplementation did not significantly alter overall microbiota diversity, while PRE host and dietary factors explained substantial compositional variance. Although global community was stable, 10/302 mucosal amplicon sequence variants (ASV) were significantly associated with the fiber supplementation, including increases in *Parabacteroides distasonis* and *Lachonospiracea* NK4A136 group. In rectal tissue, propionate and isobutyrate decreased while butyrate and acetate were unchanged. In serum, no changes were detected in acetate and propionate levels, while butyrate showed a decrease. RNA-seq identified 177 differentially expressed genes. KEGG GSEA indicated POST enrichment of metabolic/mitochondrial pathways (carbon metabolism, oxidative phosphorylation, TCA cycle) and SCFA-related pathways, while PRE-enriched pathways were dominated by immune-related signaling.

Conclusion*:* Short-term mixed fiber supplementation was associated with selective mucosal taxonomic shifts and enrichment of rectal mucosal metabolic programs, alongside changes in circulating metabolites.

Effects of High Soybean Meal Inclusion on the Gut Microbiome of Finishing Pigs

Angel Martinez, Courtney Pohlen, Joseph Halbur, Eric Weaver, Rafe Royall, Benoit St-Pierre.

Department of Animal Sciences, South Dakota State University

Soybean meal (SBM), the primary protein source in U.S. Midwest swine diets, is typically supplemented with synthetic amino acids to optimize growth. Variation in SBM inclusion can influence pig performance and gut health due to non-protein components, such as fibers that affect microbial metabolism. As new processing facilities expand SBM availability and lower its costs, understanding how high SBM inclusion impacts the gut microbiome is increasingly important. This study aimed to characterize the effects of SBM inclusion levels on swine gut bacterial communities.

546 pigs were used in a 111-day trial to evaluate diets with 1) low SBM inclusion supplemented with synthetic amino acids (5%; LowSBM) vs 2) high SBM inclusion (28%; HighSBM). To determine bacterial composition,14 fecal samples per treatment were collected for DNA extraction and 16S rRNA sequencing through PCR amplification of the V1–V3 regions from microbial DNA. Shotgun metagenomic sequencing on selected samples was performed to assess microbial functional potential. Reads were assembled with MEGAHIT, binned with CONCOCT, MaxBin2, and MetaBAT2, and annotated using DRAM.

Beta diversity by 16S indicated significant differences in bacterial composition between the two treatments (PERMANOVA, $P < 0.05$). We found specific Operational Taxonomic Units that were in higher abundance in the HighSBM ($P < 0.05$). These included Ssd-39, related to Streptococcus alactolyticus (99.5% identity match; 15% vs. 10%); Ssd-1160, related to Treponema brennaborense (88.0% identity match; 2% vs. 0.1%); and Ssd-1254, closely related to Treponema bryantii (97.0% match; 2% vs. 0.03%). Using metagenome-assembled genomes (assessment: >70% completeness; <10% contamination), annotation revealed metabolic capabilities such as polysaccharide degradation, and butyrate production.

HighSBM diets altered gut microbiome composition and metabolic potential. Short-chain fatty acids potential may be driven by bacteria specialized in SBM polysaccharide degradation. These findings indicate that SBM could play a role in shaping microbial activity in swine

---

Acute Azoxystrobin exposure induces gut microbiome disruption and hepatic transcriptional changes in zebrafish

Luoyan Duan & Christopher Gaulke A.

Department of Pathobiology University of Illinois at Urbana-Champaign

Azoxystrobin is a widely used fungicide associated with reproductive, neurological, and developmental toxicity. It can also disrupt gut microbial communities, but whether such perturbations contribute to host toxicity remains unclear. Here, we investigated the effects of acute azoxystrobin exposure across a concentration

series (5–500 mg/kg) in zebrafish, with a focus on dose- and sex-specific responses in both the host and gut microbiota.

We combined fecal 16S rRNA amplicon profiling, shotgun metagenomic sequencing, and hepatic gene expression analysis to assess links between microbiome disruption and liver responses. Azoxystrobin significantly altered microbiome composition and functional potential in a dose- and sex-dependent manner. Metagenomic profiling showed increased abundance of pathways related to xenobiotic metabolism and reduced representation of pathways involved in bacterial motility and lipopolysaccharide biosynthesis. Histologic evaluation identified biliary-region changes consistent with a ductular reaction, primarily in medium- and high-dose fish. In parallel, hepatic transcriptional programs showed signatures of stress responses, including altered expression of redox-related genes and reduced expression of genes involved in lipid and small-molecule metabolism, with sex-specific differences. Notably, hepatic transcriptional changes correlated with compositional shifts in the microbiota, suggesting coordinated host–microbiome responses to azoxystrobin exposure. Together, these findings demonstrate concurrent effects of acute azoxystrobin exposure on the gut microbiota and liver and suggest that microbiome disruption may contribute to hepatic transcriptional responses.

Impact of hybrid capture enrichment and sample preparation methods on ARG detection in wastewater

Owen, C. N. & Poretsky, R. S.

Department of Biological Sciences, University of Illinois Chicago

Wastewater receives input from the surrounding human population, allowing wastewater surveillance to reveal the presence of microbial genomic material found in that population, including antimicrobial resistance genes (ARGs). This genomic material can be characterized to some extent using metagenomic sequencing; however, ARG sequences generally have low abundances within the wastewater metagenome, making robust ARG profiling difficult without very deep sequencing. Hybrid probe-capture approaches offer a potential solution by targeting and enriching up to thousands of sequences simultaneously. Here, we characterize the performance of a hybrid probe-capture panel (Illumina RPIP) designed to enrich DNA and RNA from a set >2,000 ARGs and pathogens, comparing enriched libraries to untargeted metagenomes obtained from the same samples. Additionally, we assess how filtration and concentration pretreatments influence enrichment efficacy and ARG detection.

We analyzed primary influent from the Terrence J. O'Brien Water Reclamation Plant (Skokie, IL; n = 4) and outflow from O'Hare International Airport (Chicago, IL; n = 4). Each sample was divided into six aliquots representing different pretreatment

combinations: unfiltered, filtrate, or resuspended retentate aliquots were each concentrated using either Ceres Nanotrap Microbiome A Particles, Microbiome A+B Particles, or direct nucleic acid extraction. This yielded 48 sample/pretreatment combinations. All 48 were sequenced twice, using RPIP and untargeted metagenomic library preps.

We found that relative abundances of many ARGs were substantially higher in RPIP-enriched samples, although a subset of ARGs showed reduced representation after RPIP-enrichment. Filtration and concentration pretreatments impacted both the degree of enrichment and the overall composition of ARGs detected in enriched samples, with the greatest overall enrichment occurring in unfiltered samples concentrated with Microbiome A Particles. These results highlight how sample preparation techniques interact with hybrid capture performance and underscore the importance of method selection for wastewater-based ARG surveillance.

Combinatorial characterization of drinking water biofilm communities reveals both synergies and negative interactions affecting biomass and chloramine tolerance

Jessica Li[1], Jenna Klein[2], Ameet Pinto[3], Nina Lin[1,2]

1 Department of Microbiology & Immunology, University of Michigan,
2 Department of Chemical Engineering, University of Michigan
3 School of Civil and Environmental Engineering, Georgia Institute of Technology

Biofilms are an important and prevalent form of microbial growth in which cells attach to a surface and secrete extracellular polymeric substance (EPS). EPS protects against a variety of insults such as antibiotics, physical removal, and disinfectants; thus, biofilms can act as reservoirs of cells, including of potentially harmful species. The drinking water distribution system (DWDS) is one environment in which biofilms dominate. DWDS biofilms have been implicated in the majority of recent drinking water associated disease outbreaks in the U.S. but are understudied. Many recent studies employ only sequencing-based methods, which provide insights on community compositions and potential community functions, but confounding factors such as the presence of large amounts of extracellular DNA in the EPS and unknown levels of gene expression necessitate more culture-based functional studies.

We thus examined biofilm properties of several DWDS biofilm isolates in both monoculture and cocultures with a focus on total biomass and chloramine disinfection tolerance. We isolated ~20 strains on R2A, a low-nutrient medium used for heterotrophic plate counts at water treatment plants. These strains originated from a mix of residential showers as well as laboratory faucets. We identified several positive interactions between species that resulted in increased biofilm biomass,

measured by crystal violet staining. These positive interactions were surprisingly common, suggesting the stresses of the DWDS could select for cooperation. We then challenged select species and cocultures with chloramine and observed an increased cell survival rate, measured by CFUs, among cocultures compared to their constituent monocultures. Our results provide some of the first experimental confirmation that the positive relationship between species richness and protection against chlorine hold true for chloramine as well. In future work, we will investigate potential mechanisms for this increased chloramine tolerance with a focus on whole genome analysis and EPS characterization

---

Fertilization-conditioned soil microbiomes shape cultivar-dependent assembly of soybean nodule microbiomes

Danyang Duan[1,2], Angela Kent[1]

1. Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana-Champaign
2. Genomics and Eco-evolution of Multi-scale Symbioses (GEMS) Institute, University of Illinois, Urbana-Champaign
3. Program in Ecology, Evolution & Conservation Biology (PEEC), University of Illinois, Urbana-Champaign

Soil microbial communities serve as the primary source pool for plant-associated microbiomes, yet long-term fertilization can reshape these communities and alter their contribution to host-associated microbiome assembly. In legumes, root nodules host the most well-known plant-microbe symbioses with nitrogen-fixing rhizobia, while also harboring diverse non-rhizobial microbes that can provide additional benefits. However, how long-term fertilization shapes legume interaction with both rhizobial and non-rhizobial nodule communities via soil, and how these interactions affect plant growth, remain poorly understood.

Here, we examined how soil microbiomes conditioned by long-term fertilization influence soybean nodule microbiome assembly and soybean growth traits across two cultivars. Using soil sampled from long-term fertilized and unfertilized soils from the University of Illinois Morrow Plots, the longest-running agricultural experiments (>150 years) in the U.S. with fertilization history for over 70 years, soil slurries were inoculated into sterilized growth media for soybean. Both soil and nodule microbial communities across two soybean cultivars ('Jackson' and 'KS4895') were characterized using short- and long-read sequencing of 16S rRNA, 18S rRNA, and ITS markers, along with functional gene profiling (nifH), and we quantified the extent to which the fertilization-conditioned soil microbial community structure predicts nodule microbiome composition, and in turn, plant growth traits.

We found clear cultivar-dependent differences in soil-nodule microbiome coupling, with the two cultivars differing in both rhizobial partners and non-rhizobial

communities. Soil microbial community structure significantly explained variation in nodule microbiomes, but this relationship was primarily driven by the cultivar 'Jackson'. Moreover, relationships between nodule microbiomes and plant traits differed between cultivars. In 'Jackson', nodule weight was negatively associated with root-to-shoot ratio, indicating greater allocation to symbiotic structures (i.e., nodules) rather than structural roots and consistent with a more nodule-driven nutrient acquisition strategy; correspondingly, its nodule microbiome showed detectable fertilization effects. In contrast, the other cultivar 'KS4895' exhibited patterns consistent with root-driven growth, with no evident fertilization effect on its nodule microbiome.

Together, these results demonstrate that long-term fertilization reshapes soil microbiomes, which in turn differentially influence legume nodule microbiome assembly across cultivars and ultimately affect plant growth.

A resource allocation framework for denitrification

Dominic Cipiti, Karna Gowda

Department of Microbiology, The Ohio State University

Denitrification is a form of anaerobic respiration undertaken by facultative anaerobes to reduce nitrate (NO_3-) to nitrogen gas (N_2) in a four-step, sequential pathway. By enabling bacteria to grow under shifting redox conditions across diverse environments, denitrification plays a central role in global nitrogen cycling and regulating nitrous oxide, a potent greenhouse gas. The commonly studied bacterial denitrifiers (e.g., *Paracoccus denitrificans*, *Pseudomonas aeruginosa*) carry out the complete pathway (NO_3-→N_2). However, most naturally occurring denitrifiers perform three or fewer of these denitrification steps (partial denitrification). Energetically, complete denitrification should be favorable in nutrient-limited environments, generating more ATP per nitrogen species, yet it is observed that partial denitrifiers dominate most environments. We do not understand the selective pressures driving partial versus complete denitrification in environmental bacteria, indicating a limited understanding of denitrification biology, particularly how constraints on intracellular resource allocation give rise to the fitness of different denitrification strategies.

The goal of my project is to uncover the resource allocation biology of denitrification. We hypothesize that the interplay between carbon oxidation, respiration, and global gene expression determine denitrification phenotypes. We are using a Pseudomonas sp. soil isolate (PDM20) to uncover principles of resource allocation in denitrification. Quantifying growth and denitrification rates across a variety of nutrient conditions and mutants will serve as the foundation for generating physiological variation. RNAseq, RNA/protein ratios, and biomass yield from growth studies will be used to characterize intracellular resource allocation

across varying physiologies. These data will be used to parameterize a denitrification-specific resource allocation model. Using this model as a foundation, I will validate the predictions using genetic approaches to probe how partial denitrification may reallocate limited cellular resources to optimize bacterial growth, explaining the selective advantage of partial denitrification

---

Integrating and visualizing multiomics microbiome datasets with Metabolon's microbiome research solution

Josh Simpson and Jason Lockerfeer, Metabolon

Meaningful discovery happens when quality data, careful interpretation, and intuitive technology come together to reveal clear insights from complexity. With a single sample submission, Metabolon's Microbiome Research Solution can provide metagenomics, untargeted metabolomics, and absolute quantification of short-chain fatty acids. This comprehensive but flexible approach empowers users to tailor omics analyses to specific research objectives. Upon completion of sample analysis, users gain access to Metabolon's Microbiome Tool for intuitive data exploration and interpretation.

Built around the trusted Integrated Bioinformatics Platform (IBP), the Microbiome Tool unifies metagenomic, metabolomic, and phenotypic data within a code-less, accessible platform that simplifies complex analyses without compromising flexibility or analytical depth and rigor. From raw sequencing data to rich visualizations, the platform performs end-to-end processing without requiring manual intervention or custom engineering. Rather than focusing solely on taxonomic profiling or single omics correlations, this solution seamlessly connects microbial composition to functional metabolic activity. With this foundation in place, researchers are equipped from the outset to identify the microbes that differentiate sample groups, uncover community-level patterns, and investigate functional variation across taxonomic levels with confidence and ease. Whether the goal is mechanistic discovery or translational application, Metabolon's Microbiome Tool offers the analytical power and user-friendly experience needed to translate microbiome data into actionable knowledge. In this workshop, a Metabolon scientist will walk you through the platform and showcase real world case studies.

Genome-based identification of a novel 'Candidatus Phytoplasma' species from environmental insect samples in China

Trivellone[1], Valeria, Rendon[2], Gloria A., Hernandez[2], Alvaro H., Dietrich[1], Christopher H., & Fields[2], Christopher J.

[1] Prairie Research Institute, University of Illinois at Urbana-Champaign
[2] Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign

Natural ecosystems harbor high biodiversity and complex species (including host-pathogen) interactions. However, pathogen surveillance in such systems is rarely attempted due to low prevalence resulting from the dilution effect and because infections are often asymptomatic. Moreover, detecting a pathogen in an insect does not necessarily imply vector competence, which is difficult to demonstrate across diverse taxa. Nevertheless, insects can be effectively used as sentinels to monitor the diversity of potential pathogens circulating in natural environments.

Phytoplasmas are an ancient lineage of phloem-limited, insect-transmitted, not-yet-cultured bacteria (Mollicutes) associated with numerous plant diseases and have been primarily studied in agroecosystems, where symptomatic crops and abundant vector populations facilitate detection. Thus, most genome-based data and '*Candidatus* Phytoplasma' species descriptions derive from agroecosystems, potentially biasing our understanding of phytoplasma diversity and evolution in nature. In contrast, natural ecosystems likely represent reservoirs of phytoplasma diversity predating crop domestication, making their investigation a proactive strategy for early pathogen detection. In this study, we report the phylogenetic and genomic characterization of a phytoplasma strain detected in environmental insect samples collected in China. A near-complete genome (465,678 bp; 45 contigs; BUSCO completeness 90.6%) was reconstructed using a hybrid assembly approach combining short- and long-read data. A single nearly full-length *16S rRNA* gene (1,516 bp) showed 98.9% identity to '*Candidatus* Phytoplasma sacchari', indicating that 16S-based resolution alone is insufficient for species delineation. Genome-wide comparisons revealed low average nucleotide identity values (orthoANI: 84.77% and dDDH: 27.7% vs. '*Ca*. P. sacchari'), and phylogenetic analyses based on core genes and housekeeping markers (*secA*, *secY*, g*roEL*) supported its distinctiveness. Based on these results and current taxonomic guidelines, we propose the designation of a novel '*Candidatus* Phytoplasma' species. Our findings highlight the importance of proactive surveillance in natural ecosystems for uncovering hidden pathogen diversity prior potential spillover in agroecosystems.

Effects of oscillating temperatures on reproductive manipulating endosymbionts in spider host

Cook, E.A., Doremus, M.R.

Department of Integrated Biology, University of Illinois Urbana-Champaign

Most arthropod species are known to host a diverse array of maternally-transmitted microbial symbionts. Heritable symbionts can confer a range of host effects, with some symbionts enhancing their own vertical transmission by manipulating host reproduction to favor symbiont-transmitting females. These reproductive manipulators are widespread in arthropods but show severe sensitivity to thermal stress, which can destabilize heritable symbioses by influencing symbiont density,

transmission efficiency and the expression of conferred phenotypes. Symbiont sensitivity to thermal conditions raises questions on how heritable symbionts persist in natural populations with fluctuating temperatures and how these symbioses might respond in the context of global climate change. Yet most research on symbiont thermal sensitivity uses constant temperature regimes which do not generally reflect realistic temperature cycles. To better understand how arthropod populations and their microbial partners might respond to fluctuating temperatures, we used the spider *Mermessus fradeorum* (Linyphiidae), an emerging system for studying heritable symbioses. *M. fradeorum* variably hosts six different heritable symbionts, including a strain of the symbiont *Rickettsiella* that causes a form of reproductive manipulation called cytoplasmic incompatibility (CI). CI is a two-step form of sabotage, with the symbiont first modifying infected males to kill uninfected embryos, then rescuing embryo viability when present in the egg. We compared the effect of oscillating and constant daily temperatures on the efficacy of *Ricketsiella*-induced CI in the spider host *M. fradeorum*, hypothesizing that oscillating temperatures would mediate symbiont thermal responses by providing recovery periods at cooler temperatures. We found that despite these presumed recovery periods, *Rickettsiella* CI was significantly less effective under both constant and oscillating warm temperatures compared to cool temperatures. Our findings indicate even limited exposure to thermal stress under a fluctuating temperature scheme can destabilize host-symbiont dynamics. Oscillating temperatures should be considered when studying the effects of climate change on heritable symbioses.

Untargeted metabolomics reveals metabolic differences between symbiotic and aposymbiotic phenotypes of a facultatively symbiotic temperate coral (Astrangia poculata).

Andersson, E. R., Roberson, L. M., Schock, T. B., & Poretsky, R. S.
Biological Sciences (Ecology and Evolution), University of Illinois at Chicago

The temperate coral *Astrangia poculata* is a proposed model for studying symbioses in coral, largely due to the facultative relationship with their endosymbiotic algae (family Symbiodiniaceae) under natural conditions. By contrast, most tropical corals lose their symbionts only under stressful conditions (i.e., bleaching), a confounding factor that can make it difficult to study the role of the endosymbiotic algae in coral biology. There is evidence that both aposymbiotic (fewer algae; white color) and symbiotic (more algae, brown color) *A. poculata* phenotypes rely primarily on heterotrophy for energy. Nonetheless, these algae likely provide supplementary benefits (e.g., photosynthates, increased nutrient uptake) at the cost of an increased energetic investment to sustain larger endosymbiotic algae populations.

Although the physiology and general metabolism of aposymbiotic and symbiotic *A. poculata* have been described, there has been limited research regarding specific host-algae metabolic interactions or specific coral metabolic activities influenced by the presence or absence of the algal symbionts. To further investigate the role of the symbiotic algae in coral metabolism and nutrition, we collected complementary LC-MS- and NMR-based untargeted metabolomics data from symbiotic (n=15) and aposymbiotic (n=15) *A. poculata* collected near Woods Hole, MA.

Metabolomics results showed that betaine was the most abundant metabolite in the *A. poculata* metabolome, by an order of magnitude over the next most abundant compounds, something we have previously found in metabolomics analyses of some tropical coral species that have similar growth morphologies to *A. poculata*. Furthermore, we found increased relative abundance of betaine and trimethylamine in metabolite profiles from symbiotic compared to aposymbiotic individuals, which may have implications for osmotic regulation in their highly seasonal environment.

SIPdb: a stable isotope probing database for extending ecological interpretation to amplicon datasets

Alex Batista Trentin, Abigayle Simpson, Jeffrey A. Kimbrel, Steven J. Blazewicz, and Roland C. Wilhelm

Agronomy Department, Purdue University

High-throughput amplicon sequencing has expanded our view of microbial diversity; however, connecting sequence data to ecological function remains a central challenge. Most detected taxa lack experimentally verified metabolic traits, and reliance on culture-based characterizations or taxonomic identity as proxies for function introduces bias that compounds across studies. To address this, we compiled data from 22 published stable isotope probing (SIP) studies to build a meta-analytical database of documented isotope incorporators. From the peer-reviewed datasets, encompassing 21 labeled substrates and 14 environments, we identified ~46,000 unique incorporator ASVs across 62 phyla. Our MISIP-compliant pipeline integrates four differential abundance methods (ALDEx2, DESeq2, edgeR and limma-voom) to designate incorporators across heterogeneous SIP designs. The resulting database, paired with an interactive Shiny dashboard and BLAST interface, was designed to extend ecological interpretation to any amplicon dataset. To test whether SIPdb can inform interpretation of standard microbiome surveys, we mapped community profiles from a 1,4-dioxane degradation study onto the database (using the integrated BLAST tool). Among the 12 genera reported in the original study, five matched SIPdb incorporators (*Brevundimonas, Rhodococcus, Paenarthrobacter, Streptococcus* and *Lysobacter*), despite SIPdb containing only a single dioxane study, suggesting that the tool can help identify ecologically dominant degraders even with limited substrate coverage. Conversely,

five additional genera enriched in dioxane microcosms lacked direct dioxane matches but were identified as methane or butane incorporators, suggesting latent dioxane degradation capacity through promiscuous monooxygenase systems and demonstrating how SIPdb enables hypothesis generation beyond direct substrate matching. Additionally, SIPdb recovered eight genera not reported by the original authors but with documented dioxane incorporation in the database, highlighting its capacity to uncover overlooked candidate taxa with broader metabolic versatility. As an open-source, extensible platform, SIPdb provides a scalable framework for reverse ecology, enabling hypothesis generation and functional interpretation from standard amplicon surveys.

Whole food fibers for support of key gut bacteria for human health

Lauren Yepes, Bruce Hamaker, and Thaisa Cantu-Jungles

Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, Indiana. USA

Background and Objective: Butyrate-producing Clostridium clusters IV and XIVa are critical for maintaining gut integrity and modulating systemic inflammation. These mucosa-associated beneficial bacteria are better supported by fermentable insoluble and matrix-entrapped fibers, such as those found in plant cell walls from whole foods, rather than isolated soluble fibers such as inulin commonly added to processed foods. The objective of this study was to develop a food-grade protocol to isolate whole-food fibers and to determine whether rationally designed multi-component formulations enhance enrichment of clusters IV and XIVa relative to inulin, with planned validation in a human clinical trial.

Methods: Twenty whole foods representing five food categories were freeze-dried, milled, and enzymatically treated to reduce starch and protein while preserving fiber structure. Fiber-rich concentrates (70–95% total fiber) and their 4-,5-, and 6-component mixtures were used in standardized 24-h in vitro fecal fermentations (25 mg substrate) with pooled human inocula (n=3). Short-chain fatty acids were quantified by GC-FID, and microbial composition evaluated by 16S rRNA sequencing (V3-V4 regions).

Results: Butyrate production did not correlate with fiber quantity. Foods with similar fiber content in the same food grouping produced different butyrate levels (e.g., in fruits butyrate ranged from 3 to 6 mM). In cereals, quinoa produced higher butyrate despite lower fiber content than rice, demonstrating that structural properties, rather than fiber mass, drive fermentation outcomes. Inulin produced the highest butyrate (~12 mM), but enriched a narrower subset of butyrate-producing genera. In contrast, whole-food fibers showed substrate-specific enrichment patterns, and no single food promoted all target taxa. Mixtures produced ~7 mM butyrate and maintained broad enrichment of butyrate-

producing Clostridium. Eight combinations showed significant synergy (synergy index up to 1.13; $p < 0.01$), strongest in 4-component mixtures. A selected 4-

component formulation targeting the widest range of butyrogenic taxa, is composed of ingredients frequently present in high-synergy combinations, and it is now being tested in a clinical trial.

Conclusion: Fiber structure, rather than fiber amount alone, drives enrichment of butyrate producers, especially those from the Clostridium clusters IV and XIVa. Combining complementary whole-food fibers can produce synergistic butyrate increases, supporting an ecology-based strategy for improved prebiotic design.

A dual-color intestinal co-culture platform to evaluate host responses to inulin-derived microbial metabolites

Yuxin Wang, Peter Jackson, & Stephen R. Lindemann

Department of Food Science, Purdue University

Inulin is a soluble prebiotic fiber known to improve gut health by selectively stimulating beneficial gut bacteria, including members of genera *Bifidobacterium* and *Lactobacillus*, and enhancing the production of short-chain fatty acids (SCFAs) in the colon. However, rapid *in vitro* models to evaluate individualized gut responses to prebiotic supplementation remain limited. In this study, we developed a dual-color intestinal epithelial co-culture system consisting of GFP-labeled Caco-2 cells and mCherry-labeled HT-29 cells to evaluate host epithelial responses to microbiota-derived metabolites. Fecal water samples were collected from a volunteer before, during, and after a 6-week inulin supplementation period followed by a 2-week washout phase. Samples collected at baseline (Day 0), during supplementation (Days 4, 7, 14, 28, and 42), and during washout (Days 56, 70, and 84) were applied to the epithelial monolayer. Fecal water obtained during the supplementation period significantly increased transepithelial electrical resistance (TEER) compared with the PBS control. Day 7 samples showed the strongest effect, and washout samples reverted back to baseline. Consistently, fluorescence intensity and epithelial layer brightness increased following treatment with supplementation-phase samples. Gas chromatography analysis revealed increased SCFA levels after 7 days of inulin supplementation, which returned to baseline after cessation. HPLC analysis showed a modest increase in lactic acid levels after 4 and 28 days of inulin supplementation, whereas the dual-color co-culture exhibited higher levels of both succinic acid and lactic acid in the basolateral compartment. Together, these results demonstrate the utility of a dual-color, fluorescent co-culture model as a rapid platform for evaluating microbiota-derived metabolites and host epithelial responses to dietary prebiotic interventions.

Influence of dietary fiber consumption on stress-induced HPA axis activation

Rindels, J.E.[1], Levine, B.A.[1], Loman, B.R.[1,2]

[1]Division of Nutritional Sciences, College of ACES, University of Illinois at Urbana-Champaign
[2]Department of Animal Sciences, College of ACES, University of Illinois at Urbana-Champaign

Background: One in five U.S. adults and adolescents suffer from psychopathological conditions, primarily depression and anxiety. Chronic stress exposure is associated with the development of both anxiety- and depressive-like behaviors. The hypothalamic-pituitary-adrenal (HPA) axis is a multi-organ system that is stimulated by psychological stress exposure and concludes with the release of cortisol (corticosterone in mice (CORT)) from the zona fasciculata (ZF) of the adrenal cortex. CORT is the major stress hormone in the body, and previous data from our lab demonstrates that stress-induced CORT elevation is attenuated by consumption of dietary fiber. These effects may be due to fibers' ability to modulate the gut microbiome, particularly by increasing the abundance of *Bifidobacterium*, a bacteria known to reduce HPA axis signaling. Therefore, understanding the points along the HPA axis that are regulated by diet may provide insight into how the gut microbiome influences the development of anxiety- and depressive-like behavior.

Methods: Male and female C57BL/6 mice (age 6-8 weeks, n=8, N=128) 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 CORT via ELISA. At sacrifice, adrenals were harvested, fixed in 4% PFA, sectioned and stained using hematoxylin and eosin, and quantified. Brains were harvested, fixed in 4% PFA, flash frozen, sectioned at the PVN, and stained for CRH using immunofluorescence.

Results: Stress (S) increases CORT AUC ($p<0.0001$). S mice have larger ZF than NS mice ($p=0.0586$). S CELL and scFOS, have lower CORT, but larger ZF than S FFD ($p=0.032$). F mice have larger ZF ($p<0.0001$) and tend to have higher CORT ($p=0.19$) than M mice. S mice have higher nestlet scores than NS. Upcoming work will analyze CRH abundance in the brain and circulating ACTH.

Gut microbiome and behavior effects on Travelers' Diarrhea occurrence in a military population

Zachary Liechty[1], Arianna Baldwin[1], Blake Stamps[2], Adrienne Hatch-McChesney[3], J. Philip Karl[3], Michael Goodson[1]

[1] 711th Human Performance Wing, Air Force Research Laboratory
[2] Materials and Manufacturing Directorate, Air Force Research Laboratory
[3] Military Nutrition Division, US Army Research Institute of Environmental Medicine

Military personnel deployed to new environments are commonly exposed to pathogens which can result in impaired health and performance, resulting in reduced effectiveness. The most frequently reported health issue amongst deployed personnel is Travelers' Diarrhea. Occurrences of diarrhea can afflict over 50% of personnel during deployment, with the majority of those affected experiencing multiple episodes. While the probability of experiencing diarrhea can be somewhat attributed to lifestyle choices while deployed (i.e., handwashing, eating MREs over local economy, etc.), these lifestyle choices do not fully explain susceptibility to diarrhea-inducing pathogens. Another factor that could influence diarrhea susceptibility is the composition of the deployed personnel's gut microbiome. Microbe-microbe interactions could inhibit or enhance the activity of a diarrhea-inducing pathogen, leading the deployed personnel to potentially harbor a "resistant" or "susceptible" microbiome. To investigate this possibility, we surveyed the gut microbiomes of personnel from the 25th Combat Aviation Brigade (Wheeler Army Airfield, Hawaii) before and after participation in Exercise Salaknib 2022, an approximately 2-month deployment to the Philippines. We additionally collected information regarding the dietary and lifestyle habits prior to and during deployment. A distinct subject-dependent shift in the microbiome after deployment, with various taxonomies being significantly affected by different deployment activities. Furthermore, some of the changes in relative abundance of specific ASVs (amplicon sequencing variants) were dependent on whether the host experienced diarrhea or not, suggesting diarrhea events could have long term specific impacts on microbial composition. Several ASVs from the pre-deployment samples were found to be positively or negatively associated with experiencing diarrhea while deployed. Some of these ASVs could make promising targets to be used as a basis for prophylactic treatment in deployed personnel to reduce the incidence of diarrheal disease during and after deployment.

Disclaimer: Authors' views not official U.S. Air Force, Army or DoD policy.

Highly efficient preparation of large functional metagenomic libraries and their application to capture novel antimicrobial resistance genes

Shi Y, Bernate E, Allman H, Franck E, Crofts TS

Department of Animal Sciences, University of Illinois at Urbana-Champaign

Antimicrobial resistance poses a threat to global health and there is a need to develop innovative approaches to identify and characterize emerging resistance genes. Immediate clinical threats are well detected by direct cultivation and genetic dissection of pathogens that grow under laboratory conditions. High-throughput sequencing approaches can, in turn, detect known resistance genes in difficult or impossible to cultivate environmental isolates. However, identification of resistance threats *via* metagenomic DNA sequencing requires that detected genes have high identity to known resistance genes, meaning that novel or emerging antibiotic resistance genes are not identified. Functional metagenomic libraries circumvent the shortcomings of both cultivation- and sequencing-based approaches by expressing millions of random microbiome-derived genes in a heterologous host for functional screening or selection on a single agar plate. However, traditional functional metagenomic library construction methods require large amounts metagenomic DNA, suffer from low cloning efficiency, and often require specialized instrumentation, limiting their potential and uptake.

Here, we present and apply advances that overcome these limitations through the development and application of Mosaic Ends Tagmentation (METa) assembly, a highly efficient method for constructing functional metagenomic libraries. METa assembly leverages transposase-mediated fragmentation and homology-based assembly cloning to achieve much higher DNA-use efficiency (up to 80 Gb/ug) and library sizes (over 700 Gb). Enzymatic tagmentation also drastically reduced DNA input requirements (as low as 30 ng), enabling library construction from low-biomass microbiomes such as aquatic environments and human stool swabs. We demonstrate the utility of METa assembly by generating functional metagenomic libraries from soil, fecal (goose, mouse, and human), and aquarium microbiomes. We followed this by performing selections for antimicrobial resistance to identify novel antibiotic and disinfectant resistance genes.

Assessing the reproducibility of whole-microbial community evolution of AMR: unraveling the contributions of vertical and horizontal gene transfer

Stabryla, L., Chowdhury, N., Keenum, I., Servetas, S., Dootz, J., Hunter, M., & Jackson, S.

Department of Civil, Materials, and Environmental Engineering, University of Illinois Chicago

Propagation of antimicrobial resistance (AMR) is poorly understood within complex microbial communities. The human gut microbiome contains members of various taxa whose interactions with antibiotics may propel AMR to evolve in a myriad of ways. Understanding how, and how reproducibly, the human gut microbiome evolves under antibiotic selection pressures and other external influences, such as

exposure to extracellular AMR genes (eARGs), is critical to furthering our understanding of AMR propagation in realistic microbial communities.

Repeat passaging of ten replicates of a pooled human stool sample was carried out for 14 days with and without exposure to kanamycin and extracellular kanamycin resistance genes (via plasmid pBAV1k-T5-GFP). Over the course of the experiment, samples were characterized via culturing, digital droplet polymerase chain reaction (ddPCR), and 16S rRNA sequencing to assess the reproducibility of whole-microbial community evolution of antibiotic resistance. All ten replicates of passaged stool evolved similarly high levels of phenotypic resistance to kanamycin. In concordance with repeated passaging, the community shifted consistently across replicates and stabilized after four days of kanamycin exposure, with *Enterococcus* spp. dominating and significantly decreasing the diversity of the community. Further, the role of gene uptake (as quantified by ddPCR) was significant; however, it did not contribute to significant taxonomic changes in the microbial community nor did it have a significant impact on the level of evolved (or acquired) phenotypic resistance.

This study improves the current understanding of competing and synergistic processes that lead to evolution of a complex microbiome under antibiotic selection pressures. The approach used herein is applicable to further bench-scale studies of other types of clinically- or environmentally-relevant microbial communities (e.g., wastewater treatment plants, soil, vaginal, oral, etc.). This work is also critical to informing patient care or environmental protection efforts as it elucidates potential effects of prescribed antibiotic regimens and environmental eARG exposures.

CheckAMG and PhAuxDB enable ecosystem-scale discovery and pangenomic classification of auxiliary viral genes

James C. Kosmopoulos[1,2], Cody C. Martin[1,2], Karthik Anantharaman[1,3]

[1] Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA
[2] Microbiology Doctoral Training Program, University of Wisconsin-Madison, Madison, WI, USA
[3] Department of Integrative Biology, University of Wisconsin-Madison, Madison, WI, USA

Viruses can reprogram microbial metabolism, physiology, and gene regulation through auxiliary viral genes (AVGs), yet identifying these genes and distinguishing broadly conserved functions from ecosystem-specific ones remains a major bottleneck in viral ecology. Here, we pair a scalable AVG discovery pipeline (CheckAMG) with a viral pangenomic framework and database (PhAuxDB) to link curated AVGs to global patterns of evolutionary conservation. We applied this framework to a database of 93,000 viral genomes from global soil metagenomes.

After stringent curation to remove nonviral contamination and misleading annotations, CheckAMG identified 2,997 auxiliary metabolic genes (AMGs) spanning 486 distinct functions, along with 2,255 physiological genes and 19,269 regulatory genes. Among the AMGs, the most abundant functions were predicted to influence host sulfate reduction, denitrification, phosphate scavenging, polysaccharide degradation (non-cell-wall targets), and energy metabolism. To move beyond annotations, we grouped viral proteins into families and quantified their distribution across viral lineages, enabling statistical classification of protein families as core-like or auxiliary-like, each with distinct evolutionary dynamics. Notably, we find many virus-encoded genes with strong metabolism-related annotations split between auxiliary- and core-like patterns. These splits often occurred for families with the same annotation, suggesting that auxiliary-like families may participate in metabolic pathways in niche-specific contexts, whereas core-like families likely represent metabolic-gene homologs co-opted for broadly conserved viral functions, rather than auxiliary metabolism. Together, CheckAMG and PhAuxDB provide an ecosystem-scalable route from viral genomes to classified auxiliary- and core-like protein families, and a foundation for future studies of how viruses shape microbial function across environments.

Uncovering biosynthetic gene clusters in phage and giant virus genomes

Neha Kashyap[1], Cody Martin[1,3], James Kosmopoulos[1,3], Karthik Anantharaman[1,2]

[1] Department of Bacteriology, University of Wisconsin-Madison, Madison, WI, USA
[2] Department of Integrative Biology, University of Wisconsin-Madison, Madison, WI, USA
[3] Microbiology Doctoral Training Program, University of Wisconsin–Madison, Madison, WI, USA

Bacteria encode complex metabolic pathways that support their growth, development, and provide a competitive advantage against other microbes. This last aspect is controlled by secondary metabolites, which play a role in defense, communication, and adaptation during microbial interactions. Within the bacterial genome, colocalized genes called biosynthetic gene clusters (BGCs) encode the pathways needed to synthesize, modify, and regulate secondary metabolites. BGCs can be transferred between bacteria, giving recipient bacteria the ability to harness new biosynthetic pathways and create specialized metabolites like antimicrobials. Evidence suggests that bacteriophages may also play a small but ecologically significant role in carrying and transferring BGCs between bacteria. The ability for bacteriophages to hold BGCs in their genomes means phage infection could be beneficial to the host in such cases. Previous literature explored this phenomenon in one small, primarily host-associated genome dataset. To test this hypothesis on a dataset more representative of the global virus ecosystem, five databases – IMG/VR, GOV 2.0, GSV Atlas, Prophage-DB, and VIRE – were compiled and filtered to only

include complete and high-quality genomes as denoted by CheckV. A broad initial screen to find BGCs was conducted using both homology-based and deep-learning approaches. Preliminary findings show that BGCs are found in phage and giant virus genomes, although they are relatively rare. Many BGCs fell under the RiPP, other, or unknown categories, with unknown indicating that novel BGCs (i.e., novel biosynthetic mechanisms) may be encoded in these viral genomes. Many terpenes were also found specifically in the IMG/VR dataset. Verifying the presence and patterns of BGC distribution across ecosystems and viral and host taxa will give us deeper insight into the ability of phages to transfer and spread secondary metabolism pathways. These results also bring us closer to realizing biotechnological applications like phage-antibiotic cocktails where the antibiotic is encoded within the phage genome.

Poster Agenda

Even number posters will present at Poster Session 1 on Monday, May 11 from 4:00 – 5:30 pm. Odd number posters will present at Poster Session 2 on Tuesday, May 12 from 4:00 – 5:30 pm

Poster Number	Presentation
1	*A tale of two symbionts: identifying endosymbiont strain-level variation in a Rickettsiella induced spider manipulation* **Thea Bliss**, University of Illinois Urbana-Champaign
2	*The impact of arbuscular mycorrhizal colonization on the volatile organic compound profiles of four native Illinois prairie plants* **Ilana Zeitzer**, University of Illinois Urbana-Champaign
3	*A simple but hardy bacterial community detoxifies chlorite and breaks down benzene in the cooling water of the world's largest supercomputer for AI* **Justin Podowski**, Argonne National Laboratory
4	*Oxidative stress, inflammatory markers, and intestinal health in children prenatally exposed to Zika virus with and without microcephaly* **Hercia Martino**, Federal University of Vicosa
5	*Microbial-mediated tolerance: an underappreciated layer of plant defense against aphids* **Natalie Gadberry**, Purdue University
6	*Soil fertility and substrate chemistry impacts fungal communities in decaying wood* **Lauren Otolski**, University of Illinois Urbana-Champaign

Poster Number	Presentation
7	*Sex-specific effects of the gut microbiome on glucose regulation in obesity-associated type 1 diabetes* **Fangxin Chen**, Purdue University
8	*Metal and microbes: assessing the influence of urban land use on metal-microbe interactions throughout Chicago, Illinois* **Sierra Raglin**, University of Illinois Urbana-Champaign
9	*Mostly automated rapid genome inference environment* **Sajal Bhattarai**, Purdue University
10	*Characterizing public gut microbiome data for foundation model development and large-scale integrative analysis* **Kiersten Oderman**, University of Illinois Chicago
11	*Effects of AMF on root colonization, nutrient acquisition and plant biomass in Arabica and Robusta: a systematic review and meta-analysis* **Ankita Sawant**, School of Integrative Biology
12	*Anaerobic riboflavin degradation by human gut Lachnospiraceae* Christian Quiles Pérez, The Ohio State University
13	*Soil microbiome structure affects bacterial dispersal dynamics with implications for plant microbiome assembly* **Caroline Oldstone-Jackson**, University of Illinois Urbana-Champaign
14	*Nose-to-tail functionalization of bovine microbiomes and investigation of their role in antimicrobial resistance* **Yijun Shi**, University of Illinois Urbana-Champaign
15	*Multi-modal microbiome modeling for early warning of cold-chain microbial risk in food storage and distribution* **Masoumeh Taleshi**, Shahid Beheshti University
16	*Biochemical characterization of a GH43 enzyme involved in arabinoxylan degradation byGut microbiota* **Anurag Pujari**, Purdue University
17	*Microbial diversity–disease relationships in the human microbiome: a systematic review, meta-analysis, and synthesis of ecological mechanisms* **Fahren Zackery**, University of Illinois Urbana-Champaign

Poster Number	Presentation
18	*A short-term plant-based diet intervention supplemented with medicinal herbs altered gut microbiome and the level of biomarkers in human participants' blood samples* **Hisako Masuda**, Indiana University Kokomo
19	*75 days of the gut microbiome: how diet shapes microbial composition and metabolism of gut bacteria in mice* **Jamie Kaiser**, University of Illinois Chicago
20	*High-throughput screening for high-performing phototroph/heterotroph co-cultures via microfluidic droplets* **Jonathan Albrecht**, University of Michigan
21	*Fermentation-driven modulation of wheat fiber structure distinctly shapes gut microbiota composition and short-chain fatty acid production* **Tekmile Cankurtaran Kömürcü**, Purdue University
22	*Development and validation of a reproducible synthetic soil–plant system for controlled plant–microbe studies* **Tulio Machado**, Purdue University
23	*Survey of antimicrobial resistance genes and heavy metal tolerance genes in the Chicago area waterways* **Dakshya Karki**, University of Illinois Chicago
24	*Mass transfer constraints on microbial syntrophic metabolism revealed by flow-dependent gene expression* **Yuan Fang**, Civil and Environmental Engineering
25	*Long-term nitrogen enrichment alters spatial turnover and the relative impact of selective agents in soil bacterial communities* **Isabelle Lakis**, University of Illinois Urbana-Champaign
26	*Grain prebiotic content shapes individual gastrointestinal motility and postprandial metabolic responses* **Benjamin Levine**, University of Illinois Urbana-Champaign
27	*Anthropogenic and environmental factors affecting virus communities associated with honeybee-Varroa system* **Valeria Grande**, University of Illinois Urbana-Champaign
28	*Microbial delivery of antibodies to combat porcine respiratory disease complex* **Mitchell Bryant**, University of Illinois Urbana-Champaign

Poster Number	Presentation
29	*Microplastics and global change stressors as drivers of pathogen ecology and antimicrobial resistance in soil-food systems* **Jayita De**, University of Illinois Urbana-Champaign
30	*Consistency of community composition of Wolbachia in Penenirmus sp. feather lice (Psocodea: Ischnocera)* **Ember Clodfelter**, University of Illinois Urbana-Champaign
31	*Harnessing polyphosphate-accumulating microbes to mitigate phosphorus losses from Illinois agroecosystems* **Aislinn Geedey**, University of Illinois Urbana-Champaign
32	*Carbohydrates, cavities, and collective dynamics: how dental microbes evolve to environmental stressors upon exposure to sugar alternatives* **Madeline Shay**, University of Michigan
33	*Isolation and characterization of microbiota from human pancreatic tumors and duodenum* **Ryan Hong**, University of Michigan
34	*From cortisol to cancer-promoting signals: B. desmolans DesAB shapes gut epithelial responses* **Maayan Freiberg**, University of Illinois Urbana-Champaign
35	*Characterization of methylotrophic bacteria from retting hemp* **Kayla Sharkey**, University of Kentucky
36	*Local and global comparison of mobile genetic elements reveal gene carriage associated with incompatibility groups* **Isaiah Goertz**, University of Illinois Urbana-Champaign
37	*Effect of passion fruit seed flour (Passiflora edulis) on intestinal health and metabolic parameters in normal and obesity adult mice* **Hercia Martino**, Federal University of Vicosa
38	*Linking niche diversity to phylogenetic relatedness in freshwater bacteria assemblies* **Simon Kissel**, Northwestern University
39	*Linking belowground mutualisms to aboveground growth trends associated with the invasive legume Lespedeza cuneata and native legumes in a restored prairie habitat* **Mac Johnson**, University of Illinois Urbana-Champaign

Poster Number	Presentation
40	*Characterizing the microbiome and prevalence of Wolbachia in Culex pipiens complex and Culex restuans mosquitoes in the Midwest United States* **Becky Cloud**, University of Illinois Urbana-Champaign
41	*Targeted fiber fermentation for inflammatory and metabolic disease intervention* **Wenyan Mei**, University of Illinois Urbana-Champaign
42	*From storage to fermentation: gut microbiota preservation shapes microbial communities and metabolic outputs in vitro* **Mariana Guzman** Sanchez, Purdue University
43	*The germ-free zebrafish model: long-term viability through gamma-irradiated diets* **Lydia Okyere**, University of Illinois Urbana-Champaign
44	*Microbial eukaryotic activity on marine sinking particles: metabolic niche partitioning and links to POC flux* **Isha Kalra**, University of Southern California
45	*Viral exploitation of bacterial dormancy: entrapment, persistence, and reactivation in spore-forming microbes* **Sungyun Kang**, Indiana University
46	*The urinary tract bacterial isolates of Peptinophilus obesi and Anaerococcus sp. encode a novel 17B-HSDH* **Briawna Binion**, University of Illinois Urbana-Champaign
47	*Metagenomic analysis of the impact of wastewater discharge on the nitrogen cycle in the Chicago area water ways* **Tris Bakke**, University of Illinois Chicago
48	withdrawn
49	*Molecular detection of Powassan virus in Northern Illinois tick populations* **Aurora Marguccio**, University of Illinois Urbana-Champaign
50	*Modifications of antibody fragments for improved circulating half-life and in vivo delivery by commensal gut microbes* **Yishuo Jiang**, University of Illinois Urbana-Champaign
51	*Effect if sIgAs supplementation on in-vitro fermentation of HMOs by of breast fed and infant formula fed infant gut microbiomes* **Marcelo Guerrero**, Purdue University

Poster Number	Presentation
52	*Longitudinal analysis of spectinomycin resistance dynamics in the piglet gut microbiome* **Saralexis Torres**, University of Illinois at Urbana-Champaign
53	*Denoising genomes with a phylogenetic empirical Bayes model* **Stephanie Majernik**, Ohio State University
54	*Design of a soluble fiber mixture to promote complementary beneficial bacteria and consistent responses across individuals* **Narakorn Tanasupawimon**, Purdue University
55	*Effects of random, dilution-based subsampling of the gut microbiome on host outcomes in the American cockroach* **Sophia Stokes**, The Ohio State University
56	*Deciphering stress-response mechanisms in Caulobacter vibrioides* **Hansini Gamage Don**, University of Eastern Illinois
57	*Examining microbial fitness drivers in the legume-rhizobia mutualism* **Hannah Murray**, University of Illinois Urbana-Champaign
58	*Elucidating the hierarchy of necromass degradation in a marine sediment-derived microbiome* **Paul Lee**, University of Illinois Urbana Champaign
59	*Using ex vivo oral biofilms from saliva to identify bacteria targeted by polyphenols* **Michaela Tamamidis**, University of Illinois Chicago
60	*Ecological drivers of immune structure and viral escape in microbial populations with CRISPR* **Jiayue Yang**, University of Illinois Urbana-Champaign
61	*Core resistomes are shared across diverse One Health sources* **Nhung Do**, University of Illinois Urbana-Champaign
62	*Synergetic SCFA increase in gut microbiota in healthy and diseased individuals by fiber mixtures* **Gabriel Santiago Galeano-Garcia**, Purdue University
63	*Protein removal modifies arabinoxylan structure and fermentation kinetics* **Rajsri Raghunath**, Purdue University
64	*Establishing experimental foundations for studying biofilm invasion mechanisms in drinking water systems* **Erick J. Negrón Álvarez**, Purdue University

Poster Number	Presentation
65	*Degradation of cyanobacterial extracellular polysaccharides in microbial communities* **Toko Hisano**, Purdue University
66	*Intestinal and blood microbiota-mediated remodeling of postpartum inflammation in dairy cows* **Diana Gabriela Puerres Narvaez**, Purdue University
67	*Exploring the impact of the microbiome on bourbon fermentation efficacy and volatile production* **Cooper Samuelson**, University of Kentucky
68	*Influence of multi-strain bacterial seed inoculants on soybean phenotype, yield, and rhizosphere microbiome across five field sites* **Gabriel Price-Christenson**, Earnest Agriculture Inc.
69	*Disentangling root-associated microbes in agricultural fields in Indiana and their influence on soil phosphorus levels* **Eliazar Martinez**, Purdue University
70	*A designed prebiotic mixture reduces IBS symptoms more than probiotic treatment in a randomized crossover trial* **Anna Clapp Organski**, Purdue University
71	*Symbiont co-infection and exposure to warm temperatures modify the efficacy of Wolbachia-induced feminization in the dwarf spider, Mermessus fradeorum* **Matthew Doremus**, University of Illinois Urbana-Champaign
72	*Understanding the ocular surface microbiome and amniotic membrane proteome interaction* **Erotides Capistrano da Silva**, University of Illinois
73	*Ultra-marathon running reshapes gut microbial aromatic amino acid metabolism and disrupts barrier function* **Casey Lim**, Health and Kinesiology
74	*Precise control of outer membrane vesicles in a human gut commensal* **Mark Tarabey**, University of Illinois Urbana-Champaign
75	*Polysaccharide utilization loci link dietary fiber degradation to metabolite profiles in human gut Bacteroides* **Manal Alhawsawi**, University of Illinois Urbana Champaign
76	*Quantification of the selective potential of phenylpropanoid monomers on nitrogen-fixing populations of maize-associated bacteria* **Trever Thurgood**, Purdue University

Poster Number	Presentation
77	*Dormancy enables viral persistence in soil microbiomes* **Joy O'Brein**, Indiana University
78	*Investigating the paradoxical effect of oral aspiration on the lung microbiome using computational and in-vitro modeling* **Katy Bond**, Northwestern University
79	*Influence of Fusarium graminearum on the bacterial community in Cannabis sativa* **Austin Skenandore**, University of Kentucky
80	*Mycobiome-derived metabolites impact the pancreatic tumor microenvironment* **Dominik Awad**, University of Michigan
81	*Digital enrichment of hyperthermophilic Archaeal viruses from a natural hot spring reveals local diversification in response to host CRISPR-immunity* **Thomas Cowell**, University of Illinois
82	*Evaluate the impact of aldicarb exposure on the gut microbial communities* **Jeremiah Wanyama**, University of Illinois at Urbana-Champaign
83	*Bee bread mycobiome responses to pesticide exposure in migratory pollination systems* **Ling-Hsiu Liao**, University of Illinois Urbana-Champaign
84	*Microbial diversity and resistome profiles in suburban waterbodies revealed by long-read metagenomics* **Md Hamim Bhuiyan**, The Ohio State University
85	*Fecal microbes from male mice with liver cancer are sufficient to alter bile acid homeostasis when transferred to females* **Lucia Thompson**, University of Illinois Urbana-Champaign
86	*Gut dysbiosis promotes bacterial translocation to the pancreas worsening chronic pancreatitis* **Sajan Achi**, University of Michigan
87	*Water management restructures soil microbiomes across agricultural landscapes* **Jipeng Luo**, National Natural Science Foundation of China
88	*Genomic diversification and tailocin-mediated competition in animal-associated Xenorhabdus bacteria* **Farrah Bashey-Visser**, Indiana University

Poster Number	Presentation
89	*Effects of high soybean meal inclusion on the gut microbiome of finishing pigs* **Angel Martinez**, South Dakota State University
90	*Cryopreservation of wheat bran arabinoxylan-degrading bacterial communities and assessment of their revival through in vitro fermentation* **Miguel Alvarez**, Purdue University
91	*Native mass spectrometry implies a potential ligand of Gnavocin, a narrow-spectrum bacteriocin produced by IBD-associated bacteria Mediterraneibacter gnavus* **Ngoc Pham**, University of Illinois Chicago
92	*Exploring root flavonoids as potential biological denitrification inhibitors in the maize rhizosphere* **Holly Anderson**, University of Illinois Urbana-Champaign
93	*Empirical dynamic modeling reveals time-varying microbial interaction networks in anaerobic digestion* **Chao-Jui Chang**, National Cheng Kung University
94	*Sinorhizobium fitness on non-leguminous plant hosts* **Meghan Blaszynski**, University of Illinois
95	*An integrated gene catalogue of the zebrafish gut microbiome reveals significant homology with mammalian microbiomes* **Bushra Fazal Minhas**, University of Illinois at Urbana Champaign
96	*Differences in colonic crypt depth between sire lineages in crossbred pigs* **Maya Patel**, University of Illinois at Urbana - Champaign
97	*Comparative biofilm growth on microplastic surfaces in drinking water: polypropylene, polystyrene, and cellulose acetate* **Addrita Haque**, Purdue University
98	*Metagenomic analysis of wastewater treatment impact on microbial plastic degradation potential in the Chicago area waterways* **Megan Lucas**, University of Illinois Chicago
99	*Establishing in vivo and in vitro approaches to investigate urobiome metabolism of environmental chemicals and bladder cancer development* **Audra Crouch**, Ohio State University

Poster Number	Presentation
100	*Environment and host identity structure root-associated fungal communities of Panamanian Podocarpus spp., revealing Helotiales dominance* **Blaine Martin**, University of Illinois Urbana-Champaign
101	*A standardized toolkit for programmable protein secretion and surface display in Bacteroides thetaiotaomicron* **Yu-Hsuan Yeh**, University of Illinois Urbana-Champaign
102	*Using HIC metagenomics to characterize the diversity of the rhizobial mobilome between clover rhizosphere and nodules* **Sierra Bedwell**, University of Illinois Urbana-Champaign
103	*Metagenomic characterization of microbial communities in artificial turf fields across Chicago* **Judy Malas**, University of Illinois Chicago
104	*Exploring how a host-specific microbe modulates early gut immunity in chickens through targeted Kinome Array testing* **Caleb Skow**, Iowa State University
105	*Gestational hypertension and the placental microbiome: Dysbiosis and origins* **Marwa Saadaoui**, Sidra Medicine

Abstracts (Poster Presentations)

A Tale of Two Symbionts: Identifying endosymbiont strain-level variation in a Rickettsiella induced spider manipulation

Thea Bliss[1], Matt Doremus[1], Jen White[2]

1. Department of Entomology, University of Illinois Urbana-Champaign
2. Department of Entomology, University of Kentucky

Most arthropods host maternally transmitted endosymbiotic bacteria. Many endosymbionts manipulate host reproduction to benefit infected females and increase the likelihood of symbiont transmission. The most common form of host manipulation is cytoplasmic incompatibility (CI), in which infected males are modified to selectively kill uninfected offspring. CI symbionts can exhibit strain-level variation in the lethality of their CI phenotype, although the factors contributing to strain-level variation in CI lethality are not well understood for most CI symbionts. The spider *Mermessus fradeorum* is variably infected with six types of heritable endosymbionts, including two strains of the endosymbiont *Rickettsiella*, one of which is known to cause CI. The CI-inducing Rickettsiella (R1) dominates spider populations north of Georgia, while the second more recently identified strain (R2)

dominates spider populations south of Tennessee. Using experimental CI crosses, we tested whether the R2 strain causes CI and if CI strength differs between these two *Rickettsiella* strains. We found that crosses involving R2-infected males with uninfected female (putative CI cross) produce higher offspring mortality rates indicative of CI. We also found striking variation in the CI phenotype caused by these *Rickettsiella* strains. The R1 *Rickettsiella* causes strong CI resulting in ~80% uninfected offspring mortality, while the R2 strain causes weaker CI killing only ~20% of uninfected offspring. We also find that R2 CI-induced offspring mortality rates fall dramatically after the first egg mass. This strain level variation in CI lethality makes *M. fradeorum* a promising model system for exploring the host and symbiont factors that contribute to *Rickettsiella* CI. Our results will aid future efforts to characterize the *Rickettsiella* CI mechanism and explore the underlying factors contributing to strain-level effects on endosymbiont-associated phenotypes.

A simple but hardy bacterial community detoxifies chlorite and breaks down benzene in the cooling water of the world's largest supercomputer for AI

Justin Podowski[1], Ali Fox[1], Sarah Owens[1], Mitchell McClellan[1], Susan Coghlan[1], James J Davis[1], Rick L Stevens[1]

Data Science and Learning, Argonne National Laboratory

Microbial growth is a potentially serious concern in industrial cooling water systems, as it can lead to biofilm development which can impede water movement and reduce cooling capacity. We used deep metagenomic and metatranscriptomic sequencing of microbial communities in the cooling water of Argonne National Laboratory's Aurora Supercomputer, to understand the metabolic potential and activity of these microbes. We found a relatively simple community dominated by bacteria in *Azospira*, *Rhizorhabdus* and *Pseudomonas*, among several others. An average of nearly 50% of the total community was made up of the top 8 most abundant species, which were predicted to be aerobic, heterotrophic, and mesophilic. Transcriptional activity demonstrates that energy saving glyoxylate shunt and beta-oxidation were highly active, suggesting energy starvation and scavenging. Xenobiotic degradation of benzotriazole – a corrosion resistance additive – was evident in transcription of the full degradation pathway of benzene to acetyl-CoA and pyruvate, which likely feeds into the highly active glyoxylate shunt. Response of bacteria to the biocide chlorine dioxide is also evident from the abundant transcription of chlorite dismutase and the presence of chlorite dismutase in several minor and abundant MAGs. Autotrophic and photosynthetic pathways were not present or at very low abundance, suggesting a system which is likely dependent overall on allochthonous organic carbon. Our work provides guidance for future HPC systems management as water cooling becomes dominant, as well as insight into an emergingly prominent microbial ecosystem in the built environment which contains metabolically flexible bacteria.

Oxidative stress, inflammatory markers, and intestinal health in children prenatally exposed to Zika virus with and without microcephaly

Marcela Benevenuto Ferreira[a], Aline Ribeiro Murta[a], Glauce Dias da Costa[a], Renata Celi Lopes Toledoa , Anna Clapp Organskib , Thaisa Moro Cantu-Junglesb , Bárbara Pereira da Silvaa , Hércia Stampini Duarte Martinoa[a]

a Nutrition and Health Department. Federal University of Viçosa
b Department of Food Science. Purdue University, West Lafayette, USA

The Zika virus is an arbovirus primarily transmitted by Aedes mosquitoes and is associated with congenital infection and severe developmental outcomes, including congenital Zika syndrome. The aim of this study was to investigate the impact of Zika virus exposure on markers of inflammation, oxidative stress, and intestinal health in 81 children, five to nine years old, with and without microcephaly, from Belo Horizonte-MG, Brazil born between 2015 and 2017, divided into three groups: children exposed to the Zika virus during pregnancy with microcephaly (ZIKV+MC), exposed to the Zika virus during pregnancy without microcephaly (ZIKV-MC), and not exposed to the Zika virus during pregnancy (Control). Uric acid levels were lower in ZIKV+MC compared to both ZIKV-MC and Control. Malondialdehyde concentrations and superoxide dismutase activity decreased in ZIKV-MC relative to control, without difference with ZIKV+MC. Nitric oxide increased in ZIKV+MC compared to control. ZIKV-MC showed highest catalase activity. Albumin increased in ZIKV-MC relative to control, with no different to ZIKV+MC. Fecal pH and fecal calprotectin concentration was highest in ZIKV+MC. 16S rRNA gene sequencing was performed to assess the gut microbiota. ZIKV+MC exhibited lower alpha diversity compared to control group and showed a trend toward decreased diversity compared to ZIKV-MC ($p=0.054$). Further, while ZIKV-MC did not differ from the control group, ZIKV+MC showed altered community structure compared to both ZIKV-MC and control groups as assessed by unweighted and weighted UniFrac beta-diversity analyses. In conclusion, ZIKV+MC was associated with increased intestinal inflammation and impaired gut microbial composition, whereas ZIKV-MC improved antioxidant defenses, decreasing lipid peroxidation. Together, these results support a potential link between prenatal Zika virus exposure with microcephaly and longterm alterations in gut microbiota and host physiology.

Microbial-mediated tolerance: An underappreciated layer of plant defense against aphids

Natalie Gadberry, Laramy Enders

Department of Entomology, Purdue University

A complex dance exists between plants, their associated microbiomes, and herbivorous insects. Root microbes can prime plant systemic immune responses and modulate production of defense hormones and phytochemicals that contribute to defense against insect damage. Microbial contributions to resistance (e.g., antibiosis) are well documented, while less is known about microbial-mediated tolerance defenses (e.g., compensatory growth). Key players in the root microbiome predicted to boost tolerance are arbuscular mycorrhizal fungi (AMF), which help plants counteract insect feeding damage by increased growth and nutrient uptake. A series of behavioral choice and no-choice assays were conducted to examine the role AMF plays in modulating sorghum defenses against key aphid pests, *Schizaphis graminum* (greenbug: GB) and *Melanaphis sacchari* (sugarcane aphid: SCA), as well as changes in aphid symbiont communities. Cultivars with aphid defensive phenotypes (resistant, susceptible, unknown) were either inoculated with AMF or grown in untreated soil. Plant damage scores, biomass and aphid counts were measured at multiple time points post-infestation. In choice assays, AMF inoculated plants had similar aphid numbers but lower damage scores compared to untreated controls. AMF also improved biomass of infested plants, but effects varied across cultivars. In no-choice assays, damage worsened quicker with SCA compared to GB, although population sizes were comparable. Initial analysis suggests AMF-mediated plant defenses (resistance or tolerance) vary across cultivars and aphid species. Investigation of AMF impacts on aphid symbiont abundances will be discussed. Sorghum is an important crop grown worldwide, therefore understanding interactions between AMF, plant defenses and aphid damage, is critical for developing novel pest management tactics.

Soil fertility and substrate chemistry impacts fungal communities in decaying wood

Otolski, L. C., Dalling, J.

Department of Integrative Biology, University of Illinois Urbana-Champaign

Wood decomposition is a crucial process with implications for nutrient release and carbon cycling. Numerous factors – including wood density, nutrient concentrations, and substrate location – influence decay rates, often through altering the ability of microbial decomposers to access sugars and crucial nutrients in the wood.

We explored how wood chemistry and soil nutrient levels impacted decomposition rates and fungal communities in a common garden experiment in Fortuna Forest Reserve, Panama. Branch wood from eighteen tropical tree species was allowed to decompose for two years in two adjacent sites (one with low-fertility soil and one with high-fertility soil). Following this, sawdust was collected for DNA extraction and inductively coupled plasma optical emission spectroscopy (ICP-OES) for nutrient quantification. Fungal communities were determined for pre-decay and post-decay wood through amplification with ITS4 primers and sequencing on an Illumina MiSeq i100.

We found wood species, plot fertility, and time to all impact fungal communities in the wood. Fungal community diversity increased as decay progressed, with the dominant class shifting from only Sordariomycetes pre-decay to include Agaricomycetes post-decay. Decomposition was higher in the high-fertility site, and fungal communities across wood samples were more similar within a site compared to between sites. The proportion of Ascomycota OTUs was also much higher in the high-fertility site samples compared to the low-fertility site samples, although the magnitude of difference varied by wood species.

Sex-Specific Effects of the Gut Microbiome on Glucose Regulation in Obesity-Associated Type 1 Diabetes

Fangxin Chen[1], Lavanya Reddivari[2], Heba M. Ismail[3], Tzu-Wen L. Cross[1].

[1]Department of Nutrition Science, Purdue University, West Lafayette, IN 47907, USA
[2]Department of Food Science, Purdue University, West Lafayette, IN 47907, USA
[3]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Obesity is increasingly prevalent in youth with type 1 diabetes (T1D) and may exacerbate metabolic dysfunction, glycemic control, and insulin resistance. Our previous work showed lower levels of microbial metabolites, such as secondary bile acids and acetic acid, in obese T1D youth, compared to lean counterparts. However, the role of the gut microbiome in the weight gain and obesity-associated metabolic dysregulation in the onset of T1D in youth remains unknown.

OBJECTIVE: To determine the modulatory role of gut microbiome in the pathogenesis of obesity in youth with T1D.

METHODS: Five- to six-week-old male and female C57BL/6J germ-free mice were colonized with fecal microbiota from sex-matched lean or obese human donors via fecal microbiota transplant. Six weeks after the colonization, an oral glucose tolerance test (OGTT) was performed to assess glucose regulation. One week after OGTT, mice were euthanized, and tissues were collected. Pancreatic insulin levels were measured by ELISA.

RESULTS: Body weight and total fat mass were not significantly different between mice receiving microbiota from lean and obese donors in either sex. In males, mice that received obese donor microbiota exhibited a higher area under the curve for blood glucose during OGTT and higher pancreatic insulin levels than those receiving lean donor microbiota. In females, mice receiving obese donor microbiota exhibited a lower area under the curve for blood glucose during OGTT compared with mice that received lean donor microbiota. The pancreatic insulin levels did not differ between groups in females.

CONCLUSION: In both sexes, gut microbiota from lean and obese donors influenced glucose metabolism despite comparable body weight and adiposity. However, the pattern of the differences in glucose response was opposite in females and males. Further characterization of microbial metabolite profile is needed to elucidate the functional impacts of the gut microbiota on obesity-associated metabolic phenotypes in T1D.

Metal and microbes: Assessing the influence of urban land use on metal-microbe interactions throughout Chicago, Illinois

Sierra S. Raglin[1], Andrew Margenot[2], Marynia Kolak[3], Angela D. Kent[4,5]

1. Infection Genomics for One Health, Carl R. Woese Institute for Genomics Biology, University of Illinois at Urbana-Champaign
2. Department of Crop Sciences, College of Agricultural, Consumer, and Environmental Sciences, University of Illinois at Urbana-Champaign
3. Geography and Geographic Information Sciences, School of Earth, Society, and Environment, University of Illinois at Urbana-Champaign
4. Program for Ecology, Evolution, and Conservation Biology, School of Integrative Biology, University of Illinois at Urbana-Champaign
5. Department of Natural Resources and Environmental Sciences, College of Agricultural, Consumer, and Environmental Sciences, University of Illinois at Urbana-Champaign

Antimicrobial resistance (AMR) promotes microbiome adaptation to stress, but the contribution of human land use history to antibiotic resistance genes (ARG) evolution is not well characterized. Urban soils have undergone substantial land development, resulting in spatially heterogeneous metal deposition that may drive AMR evolution as microbes adapt to metal stress. Our overall objective is to elucidate the human socioecological drivers of soil microbiome AMR functions within Chicago, Illinois. We hypothesize that spatial heterogeneity in metal contamination will correlate with parallel patterns in ARG distribution within the Chicago soil microbiome and that these patterns are influenced by social determinants of health that influence metal deposition. We sequenced soil samples (N = 300) collected throughout Chicago, Illinois, using Illumina shotgun

metagenomics (24 billion reads) to characterize the microbiome and the diversity of ARGs, using the AMR++ pipeline, incorporating microbial ARG data with metal analyses (Cu, Zn, Pb, Co, Cd, etc.) and social determinants of health (i.e. traffic intensity, urban tree greenness, etc.), to identify signatures of land use within the soil microbiome. Preliminary analyses suggest that multidrug resistance and metal-resistance efflux pumps significantly contribute to the resistome composition and are influenced by Fe and Al, as well as trace metals such as V and Be. Land use data is currently being processed and will be incorporated to identify the landscape-scale patterns in land use contributing to metal-microbe interactions.

Mostly automated rapid genome inference environment

Bhattarai, S[a], Deemer, D.[b], and Lindemann, S.R.[a]

[a] Department of Food Science, Purdue University, 745 Agricultural Mall Drive, West Lafayette, IN 47907. Email: bhattar3@purdue.edu
[b] Rosen Center for Advanced Computing, Purdue University, 101 Foundry Drive, West Lafayette, IN 47907 ddeemer@purdue.edu

Prokaryotic genome sequencing has advanced significantly over the last two decades, shifting the core challenge to functional interpretation of the available data. Although current annotation and curation efforts provide information in layers, they often operate in isolation, producing heterogeneous outputs with distinct implicit logic. Though some tools are able to compile multi-layered evidence, these layers lack consensus; this occurs largely because of the lack of genome context built within the annotation logic, leading to either unresolved gene functions, or in many cases, misannotations. Furthermore, lack of auditable provenance makes it difficult to handle disagreements and uncertainty, prevent annotation redundancy, and reconcile data across varying assumptions of disparate tools.

Here, we present MARGIE (Mostly Automated Rapid Genome Inference Environment), a genome-centric, context-aware framework designed to make evidence integration explicit, interpretable, and extensible. MARGIE begins with gene prediction, aggregates functional evidence from multiple specialized databases, and incorporates genomic context directly into the annotation pipeline to give a unified, structured human readable output. To represent evidence breadth and agreement, MARGIE generates a concordance score for every gene. The scoring system integrates large-scale bacterial datasets with the analytical capabilities of a large language model (LLM). One of the important advantages of this approach lies in the identification of biologically coherent gene clusters even when homology-based evidence is weak. Ultimately, MARGIE provides comprehensive, human-interpretable information with traceable logic. By preventing redundant annotation and leaving final functional inferences to the

user, the pipeline remains "mostly automated". For convenience, MARGIE pipeline can be run via a web-hosted app. This provides a scalable, reproducible foundation for interpreting gut bacterial genomes.

Characterizing public gut microbiome data for foundation model development and large-scale integrative analysis

Kiersten Oderman, Tarak Nandi, Ravi Madduri, Joseph Walker, Jarrad Hampton-Marcell

Department of Biological Sciences, University of Chicago

Background: Publicly available microbiome sequencing data have expanded rapidly, creating new opportunities for large-scale biological discovery and computational modeling. However, despite the volume of available data, many archived gut microbiome datasets remain poorly structured and insufficiently annotated for interoperable reuse and downstream modeling. In this study, we examined gut microbiome sequencing metadata from the NCBI Short Read Archive (SRA) to assess dataset organization, metadata completeness, and suitability for integrative analyses.

Methods: A keyword-based search strategy identified 636,338 gut microbiome-related BioSample records in the NCBI SRA. Records were filtered and grouped into human-associated, mouse-associated, and generic gut metagenome cohorts based on organism annotations and available metadata. Metadata fields were analyzed to evaluate host representation, dataset structure, sequencing platform usage, temporal trends, and completeness of core descriptors. A structural topic model was applied to publications linked to archived sequencing datasets to characterize changes in scientific focus over time and across sequencing technologies.

Results: Human-associated datasets accounted for 55.17% of records, while mouse-associated datasets represented 16.56%; the remaining 28.28% were labeled generically as “gut metagenome,” limiting reliable host attribution. Sequencing output increased substantially over time, with rising sequencing depth and strong technological consolidation, as 91.82% of records were generated on Illumina platforms. Key metadata fields were inconsistently reported; for example, library name was missing in 10.68% of human records. Topic modeling identified ten dominant research themes, including mouse immunology and tissue response, microbial metabolism, infant microbiome development, comparative microbiomes, antibiotic resistance, host molecular interactions, core gut microbiota composition, diet–microbiome interactions, human cohort studies, and exercise-related metabolic health.

Conclusion: Public gut microbiome datasets are extensive, but inconsistent host labeling, incomplete metadata, and uneven structure remain major barriers to large-scale integration and microbiome foundation model development. These findings provide a basis for future harmonization efforts and improving AI readiness across public microbiome sequencing archives.

Effects of AMF on root colonization, nutrient acquisition and plant biomass in Arabica and Robusta: a systematic review and meta-analysis

Ankita A. Sawant[1], Andrew J. Margenot[2]

[1]School of Integrative Biology, University of Illinois, Urbana-Champaig
[2]Department of Crop Sciences, University of Illinois, Urbana-Champaign

Arbuscular mycorrhizal fungi (AMF) can form symbiotic associations with *Coffea arabica* and *C. canephora*, yet the interaction between AMF and coffee across varying biological, environmental, and management contexts still remain unclear. We conducted a systematic review and meta-analysis to assess degree of AMF colonization and community composition in coffee roots, quantify the effect of AMF on coffee plant nutrient acquisition, growth and cherry biomass. To address these objectives, the following factors were analyzed: 1) Arabica varieties, 2) presence or absence of AMF via exclusion experiments, and inoculum type, 3) fertilization practices and 4) edaphoclimatic conditions. Studies were identified using Web of Science and screened for the inclusion of, 1) coffee species and varieties, 2) AMF colonization in coffee roots, 3) effect of AMF on plant growth and nutrient uptake in coffee, 4) description of AMF species identified in coffee roots, and 5) inoculation type in experiments comparing AMF presence or enrichment relative to absence or scarcity. A total of 21 studies comprising 246 observations were collected. Only one study evaluated Robusta, precluding comparison between coffee species. Across studies, Glomeraceae and Acaulosporaceae were reported to be dominant families of AMF colonizing Arabica. There was no effect of MAT and MAP on AMF colonization across studies and study scale. AMF colonization in Arabica increased plant height (106%), leaf area (160%), above- (75%) and belowground (72%) and total biomass (145%). These findings highlight the role of AMF in promoting plant growth in Arabica, with implications for fertilization strategies and practices such as AMF inoculation to improve coffee productivity. However, the lack of studies on Robusta indicates the need for further research to understand AMF interactions across this commercially important coffee species.

Anaerobic riboflavin degradation by human gut Lachnospiraceae

Christian J. Quiles-Pérez, Alexandria Olzak, Aminata Fofana, Kamal Deep, Calissa Carlisle, Emily Bradley, Kathryn Kananen, Connor Skaggs, Justin A. North, Patrick H. Bradley

Department of Microbiology, The Ohio State University

Vitamins mediate a web of cross-feeding interactions in the human gut. Many Gram-positive gut microbes, in particular, are predicted to be vitamin auxotrophs. Previous studies of these microbes, however, have tended to use rich media, precluding controlled perturbations of low abundance nutrients. We tested the ability of diverse Lachnospiraceae, the most common Gram-positives in the gut, to grow on a chemically defined medium. Even though this medium contained riboflavin, we found that predicted riboflavin auxotrophs grew poorly, including the bile metabolizer Clostridium scindens. High-dose riboflavin supplementation enhanced growth, but also revealed that surprisingly, C. scindens catabolizes riboflavin into lumichrome, making it the first reported anaerobe to do so. The only previously described catabolic pathway for riboflavin requires oxygen and has no homologs in C. scindens. In high-dose riboflavin, a single gene neighborhood with an aldolase, oxidoreductases, and a riboflavin kinase/adenylyltransferase was upregulated, suggesting an alternative anaerobic degradation or overflow pathway. Similar neighborhoods were detected in several other Lachnospiraceae, including Faecalicatena fissicatena, the only other anaerobe reported to degrade riboflavin. Reanalysis of published metabolomic data showed that in vivo, both riboflavin and lumichrome were more abundant in colonized (vs. germ-free) mouse ceca, and that in vitro, Lachnospiraceae isolates depleted riboflavin while certain Gram-negative isolates overproduced it. These results demonstrate that a member of the Lachnospiraceae can anaerobically convert an essential B vitamin into lumichrome, a molecule recently shown to have anti-inflammatory properties. Vitamin catabolism may both structure cross-feeding interactions in the gut and affect host health.

Soil microbiome structure affects bacterial dispersal dynamics with implications for plant microbiome assembly

Oldstone-Jackson, C., Mann, G., Yuengling, E., Martin, L., Rana, A., and Bergelson, J.

Plant Biology/Institute for Genomic Biology, University of Illinois, Urbana-Champaign

Dispersal is a key process in microbiome assembly, yet little is known about bacterial dispersal dynamics and their effects on plant microbiome assembly. Here,

using a closed, peat-based microcosm and a complex, bacterial synthetic community, we show that requiring bacteria to disperse through less than six centimeters of soil has long-lasting effects on *Arabidopsis thaliana* microbiome structure. Strikingly, this is in part because soil microbiome structure alters the dispersal dynamics of bacteria; a competitive member of the leaf microbiome was able to disperse through the microcosm in isolation, but dispersal limited in the presence of the synthetic community. When the synthetic community was present, this *Acidovorax* species failed to effectively join *A. thaliana* leaf communities unless dispersal requirements were artificially bypassed, despite persisting in soil communities less than 6 cm away from the plants for at least eight weeks. This highlights the need to explore if these "biological barriers" to bacterial dispersal exist in nature. If so, developing strategies to overcome these barriers may improve the outcomes of intentional plant microbiome manipulations for ecological and agricultural purposes.

Nose-to-tail functionalization of bovine microbiomes and investigation of their role in antimicrobial resistance

Shi, Y., Berkmeyer, P., McCann, J. C., & Crofts, T.S.

Department of Animal Sciences University of Illinois, Urbana-Champaign

Antimicrobial resistance in livestock-associated microbiomes is becoming a challenge for animal healthcare and welfare. The widespread use of antimicrobials in livestock creates selective pressure to increase resistance genes. The ability of antimicrobial resistance genes to transfer between bacteria and microbiomes further emphasizes this threat, which may potentially extend into human clinical practice. To address this problem, we work to identify novel antimicrobial resistance genes in cattle microbiomes.

Traditional sequence-based approaches to identify antibiotic resistant genes are highly successful at identifying already known resistance genes, but these approaches are limited by their reliance on gene databases. One method to circumvent this limitation is the application of functional metagenomic selections. This method, in which metagenomic DNA extracted from microbial communities is cloned and expressed in a heterologous host to make a functional metagenomic library, uses changes in drug susceptibility to identify metagenomic DNA fragments that contain antimicrobial resistance genes. This method is powerful because it does not rely on sequenced resistance gene databases and does not require cultivation of recalcitrant environmental isolates. Here, we will use our METa assembly method to prepare functional metagenomic libraries from metagenomic DNA collected from regions of feedlot cattle digestive tracts (rumen, jejunum, cecum, and feces), providing a representation of microbial diversity across cattle. These libraries will be selected for resistance to antibiotics commonly used in cattle

agriculture, such as virginiamycin, tylosin, and tilmicosin, allowing us to identify novel resistance threats independent of prior sequence or cultivation data.

As an ongoing study, we expect this work to grant deep insights into the cattle resistome and provide a more comprehensive understanding of the distribution, dynamics, and transmission of antimicrobial resistance in these economically important animals.

Multi-modal microbiome modeling for early warning of cold-chain microbial risk in food storage and distribution

Masoumeh Taleshi[1, *]

[1, *]: Graduated MSs, Shahid Beheshti University, Faculty of Biosciences and Technology, Tehran, Iran, qazaleh.taleshi1375@gmail.com, Phone Number: +989352619417

Food loss and foodborne risk are strongly shaped by microbial dynamics along the cold chain, yet routine quality control (QC) is typically reactive (end-point counts) rather than predictive. A major challenge is that microbial composition is high-dimensional and compositional, while the operational drivers of microbial growth (temperature excursions, humidity, dwell time, handling events) are time-dependent and facility-specific. Here, we propose an interpretable, multi-modal machine learning pipeline that forecasts microbial non-compliance risk in food storage and distribution settings, linking environmental conditions (habitat) to microbial communities (harvest) with clear implications for downstream host health (OneHealth). We use a multi-source dataset consisting of: (i) routine QC microbiology (total viable count, coliforms, yeasts/molds; pass/fail against internal or regulatory thresholds), (ii) continuous environmental logs from storage zones (temperature/humidity time series and excursion statistics), and (iii) 16S rRNA amplicon profiles from periodic swabs of food-contact and near-contact surfaces and/or product surfaces. Microbiome tables are processed with standard QC and compositional transforms (CLR), then embedded using a variational autoencoder (VAE) to obtain low-dimensional community states. Environmental time series are encoded using a 1D CNN over windowed sensor streams. We benchmark logistic regression and LightGBM against two deep architectures: VAE-MLP (microbiome-only) and a late-fusion VAE+CNN model (microbiome + sensors + metadata). Facility- and batch-aware cross-validation is used to reduce leakage and over-optimism. In a representative pilot evaluation, the fusion model achieved AUROC = 0.88 ± 0.03, macro-F1 = 0.80 ± 0.04, and balanced accuracy = 0.81 ± 0.03, improving over the strongest non-deep baseline (LightGBM: AUROC = 0.83 ± 0.04, macro-F1 = 0.74 ± 0.05). Model explanations (SHAP for LightGBM; integrated gradients for deep fusion) consistently prioritized temperature-above-threshold duration, humidity variability, and VAE-derived community shifts enriched in psychrotrophic spoilage-

associated taxa (e.g., Pseudomonadaceae) alongside reductions in putatively protective fermenters. These results support a practical, explainable early-warning layer for cold-chain QC that can guide targeted interventions, reduce waste, and strengthen OneHealth-aligned microbial monitoring.

Biochemical characterization of a GH43 enzyme involved in arabinoxylan degradation by gut microbiota

Anurag Pujari, Dr. Stephen Lindemann

Food Science Department, Purdue University

Arabinoxylans (AXs) are abundant hemicelluloses in cereal grains and a major component of dietary fiber reaching the human large intestine. AX consists of a β-1,4-linked xylose backbone with substitutions including arabinose and glucuronic acid, requiring diverse carbohydrate-active enzymes (CAZymes) for complete deconstruction. Gut bacteria, particularly members of the Bacteroidota, encode polysaccharide utilization loci (PULs) that coordinate the enzymatic breakdown of complex glycans. Glycoside hydrolase family 43 (GH43) enzymes are frequently associated with xylan degradation, where they contribute to debranching and processing of arabinoxylan-derived oligosaccharides. However, the substrate preferences of many PUL-encoded GH43 enzymes remain poorly characterized.

In this study, we report the preliminary biochemical characterization of a recombinant GH43 enzyme derived from a human gut bacterium. The enzyme was heterologously expressed in *Escherichia coli* and its activity against arabinoxylan was assessed using crude cell lysate. Reactions were carried out in HEPES buffer at 37°C, and time-course aliquots were analyzed by high-pressure size-exclusion chromatography (HPSEC) with refractive index detection using PolySep GFC-P columns in tandem. Chromatographic profiles revealed a progressive reduction in the high-molecular-weight arabinoxylan peak over time, with concurrent appearance of lower-molecular-weight species, consistent with enzymatic hydrolysis. Ongoing work includes purification of the recombinant enzyme by immobilized metal affinity chromatography, assessment of substrate specificity using structurally distinct arabinoxylans, and quantification of hydrolysis products by reducing sugar assays.

These findings contribute to our understanding of how individual CAZymes within PULs participate in dietary fiber degradation, with implications for microbiome-mediated fiber metabolism and host health.

Microbial diversity–disease relationships in the human microbiome: a systematic review, meta-analysis, and synthesis of ecological mechanisms

Fahren R. Zackery, Eliza M. Grames, & Brian F. Allan

Program in Ecology, Evolution, and Conservation Biology, University of Illinois at Urbana-Champaign

Microbial diversity plays a key role in shaping disease dynamics in ecological systems, yet its influence on human health outcomes remains poorly understood. The human body hosts diverse microbial communities across multiple regions, and although many studies quantify microbial diversity, the direction and mechanisms of diversity–disease relationships are not well defined. My research addresses this gap by integrating a meta-analysis, systematic review, and mechanistic synthesis to investigate how microbial diversity relates to health outcomes across body regions. In preliminary work, I conducted a large-scale meta-analysis identifying 57,594 articles, from which 358 met inclusion criteria, yielding 583 diversity–disease relationships. Results showed that the gastrointestinal, respiratory, oral, and urogenital microbiomes consistently exhibit negative diversity–disease relationships, where decreased microbial diversity is associated with poorer health outcomes, while the skin microbiome showed a neutral relationship.

To better understand the mechanisms underlying these patterns, I performed a systematic review of 50 studies and used concept mapping to extract and visualize hypothesized pathways linking microbial diversity to disease outcomes. Building on this, I am synthesizing ecological mechanisms using tools such as HypoWeavr to construct hypothesis networks and identify recurring pathways, including colonization resistance, competitor regulation, immune enhancement, pathogen inhibition, and inhibitory metabolites. For example, decreased microbial diversity may reduce colonization resistance, leading to pathogen overgrowth and increased disease severity. By coding these pathways across studies, this work will generate a unified conceptual framework linking microbial diversity to human health outcomes across body regions and identify key microbial processes that may serve as targets for future medical interventions and disease prevention strategies.

A short-term plant-based diet intervention supplemented with medicinal herbs altered gut microbiome and the level of biomarkers in human participants' blood samples

Author: Kim Mossburg & Hisako Masuda

Department of Chemistry, Indiana University Kokomo

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. As a part of an evidence-based community health improvement initiative, we recruited human participants and investigated the impacts of ginger and curcumin on their health metrics and gut microbiota. Healthy adult participants between the ages of 18 and 45 were asked to partake in ginger and curcumin supplements daily for a period of one week or one month. 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 changed 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.

---

75 days of the gut microbiome: how diet shapes microbial composition and metabolism of gut bacteria in mice

Jamie Kaiser, Garry Lewis, Judy Malas, and Jarrad Hampton-Marc

Department of Biological Sciences, University of Illinois Chicago

Diet plays a significant role in altering body composition and shaping the gut microbiome. Diets high in carbohydrates have been shown to produce significant increases in weight and fat mass, whereas high-protein diets have shown to shown to increase lean mass. However, the extent to which the gut microbiome acts as a mediator between diet and body composition remains elusive. In this project, we compare the effects of high carb (HC), high protein (HP), and standard chow (SC) diets on gut microbiome composition and body composition (defined by proportions of fat and fat-free mass) in a rigorous 75-day longitudinal mouse study. Mice were given either HC, HP, or SC diets and were weighed daily to track overall body mass. Fecal samples were collected daily and analyzed via 16S rRNA amplicon sequencing to determine microbiome composition. Weekly nuclear magnetic resonance (NMR) body composition scans were performed to measure fat and fat free mass. Following experimentation, microbiomes were analyzed for diversity, stability, and predictive power using supervised machine learning. Mice fed the HC diet had more fat mass and less fat-free mass than those given the SC diets ($p=0.012$ for both), and HP diet mice showed greater fat mass than SC diet mice ($p=0.022$) at week 11. Shifts in body and microbiome composition occur rapidly following the introduction of a new diet. All microbiomes at the beginning of the

study clustered together, but the SC shifted away from the HC and HP diets over time. Taxa capable of fiber degradation appeared readily in SC diet, amino acid fermenters defined the HP diet, and the HC diet displayed metabolic flexibility—providing evidence that each diet results in defined microbial communities with distinct functions. Current work is focused on exploring body composition as a predictor of microbiome composition and determining key taxa responsible for responsiveness to dietary changes. Finally, generating predictive machine learning models will enable us to test in silico microbiome response not only to diet, but also to factors such as probiotics and xenobiotics.

High-throughput screening for high-performing phototroph/heterotroph co-cultures via microfluidic droplets

Albrecht, J. A., Hayashi, C., & Lin, X. N.

Department of Chemical Engineering, University of Michigan - Ann Arbor

Co-cultures of phototrophs and heterotrophs are found naturally in the environment and are known to exhibit mutualistic interactions under proper conditions. Engineers have created novel consortia for many use cases including industrial production of valuable chemicals and remediation of chemically contaminated sites. When generating new-to-nature consortia, it sometimes becomes an issue to manually test all the combinations of lab generated mutants or environmental isolates as the combinatorial space expands. Here we present a high-throughput screening approach for identifying positive phototroph-heterotroph interactions through co-localization inside microfluidic droplets.

To benchmark this pipeline, we are utilizing co-cultures of sucrose-secreting cyanobacteria with sucrose-catabolizing heterotrophs as a model system. We have recently identified that there is a large growth difference in the cyanobacteria partner when they are co-cultured with C. glutamicum vs. the E. coli K-12 strain. In this poster we lay out our pipeline in detail starting with droplet generation, followed by incubation, sorting, and finally, confirmation of enrichment. We also compare flask, 48- well, and droplet monoculture and co-culture growth to show how results agree or differ between incubation regimes. This soon-to-be-published work represents the first reported use of natural chlorophyll a fluorescence for FACS based droplet sorting of microbial consortia. We implement this pipeline with a full spectrum FACS (Cytek Aurora CS). We have also developed a method to interface the 96-well plate sorting output with a 1-well agar plate for easy post-FACS droplet breakage and colony outgrowth for post-sorting experiments. The pipeline we have demonstrated here can be adapted and extended by other labs to study numerous other microbial photosynthetic ecosystems and explore otherwise inaccessible search spaces

Fermentation-driven modulation of wheat fiber structure distinctly shapes gut microbiota composition and short-chain fatty acid production

Tekmile C. Komurcu[a,b], Miguel A. Alvarez Gonzales[b], Stephen R. Lindemann[b], Yunus E. Tuncil[a]

[a]Department of Food Engineering, Necmettin Erbakan University, Konya, Türkiye
[b]Department of Food Science, Purdue University, West Lafayette, IN, USA

The increasing prevalence of metabolic disorders is closely linked to the excessive consumption of refined foods and insufficient dietary fiber intake. Whole grains, particularly ancient wheat varieties, are considered important sources of dietary fiber that can beneficially regulate the gut microbiota. However, the extent to which biological processing techniques such as fermentation alter the fiber structure and, thus, affect microbial metabolism is still not fully understood. This study aimed to evaluate the effects of fermentation on the structural properties of dietary fibers of modern (*Tr. aestivum, Tr. durum* and *Tr. compactum*) and ancient (*Tr. monococcum, Tr. dicoccum* and *Tr. spelta*) wheat varieties and to determine how these changes affect gut microbiota composition and short-chain fatty acid (SCFA) production. Wheat samples were fermented with *Saccharomyces cerevisiae* (6% w/w, corresponding to approximately 6 × 10^7 CFU/g of wheat) for 6 h at 30 °C, and subsequently subjected to *in vitro* upper gastrointestinal digestion. Structural changes in dietary fibers were assessed by monosaccharide analysis. The fermentability of digested fractions was assessed through *in vitro* fecal fermentation analysis during which microbial SCFAs (acetate, propionate, and butyrate) and microbial community compositions were determined using gas chromatography and 16S amplicon sequencing. Fermentation significantly altered the structures of wheat fibers, with fermented varieties revealing significantly ($P < 0.05$) higher glucose, but lower arabinose and xylose contents. SCFA production generally increased over time in all samples, and significant differences among wheat varieties indicate genotype-mediated variations in microbial fermentation. Additionally, significant microbial community variations were observed depending on wheat genotype and processing conditions, as reflected by shifts in key taxa such as *Butyrivibrio crossotus* and *Parabacteroides distasonis*, suggesting that both factors play a critical role in shaping gut microbiota responses. These findings demonstrate that fermentation is an effective strategy to enhance the microbiome-modulating potential of wheat-based substrates. The results may contribute to a better understanding of how processing-induced changes in dietary fiber structure translate into functional outcomes in the gut ecosystem.

Survey of antimicrobial resistance genes and heavy metal tolerance genes in the Chicago area waterways

Dakshya Karki, Rachel Poretsky

Biological Sciences Department, University of Illinois Chicago

A growing global health concern, antibiotic resistance is affected by increased antibiotic use and improper disposal, and further exacerbated by other pollutants such as heavy metals, microplastics, and fertilizers. The presence of antimicrobial resistance genes (ARGs) is often correlated with heavy metal tolerance genes (HMTGs) in bacteria which is concerning as heavy metals can enter the aquatic environment through natural sources like leaching or erosion and increasingly through anthropogenic use in fertilizers, hospitals, and industry. The Chicago Area Waterways (CAWS) includes several channels and rivers in Chicago and is important for recreation, shipping, and as the receiving water body for treated wastewater and stormflow; it receives 1.3 billion gallons on average of treated wastewater from the three largest Chicago area wastewater treatment plants (WWTPs). Additionally, several active and inactive steel mills are located in close proximity to the waterways, adding a risk of metal discharge.

However, little is known about which resistance genes are present in the CAWS system that can create an environment allowing bacteria to exchange ARGs and MRGs posing a public health risk by creating resistant pathogenic bacteria. We collected water and sediment samples bimonthly for a year upstream and downstream from the O'Brien, Stickney, and Calumet WWTPs, including raw influent and effluent wastewater. Using metagenomic sequence analysis we aimed to determine what resistance genes we can find and how they are correlated. We also used data from the influent and effluent wastewater to compare resistance gene abundance at each WWTP using different tertiary treatment methods. Initial findings show a difference in AGRs and MGRs upstream and downstream from the WWTPs. Similarly, for the treatment variations, seeing the difference in gene abundance in the treated effluent will be important in understanding efficiency of different wastewater treatment methods.

Mass transfer constraints on microbial syntrophic metabolism revealed by flow-dependent gene expression

Yuan Fang, Ran Mei

Civil and Environmental Engineering, University of Illinois Urbana-Champaign

Syntrophy is the obligate metabolic interdependence between microorganisms and plays a central role in carbon cycling in anaerobic microbiomes such as sediments and engineered bioreactors. Syntrophic microorganisms exchange diffusible electron carriers (i.e., hydrogen and formate) to perform reactions that neither organism can accomplish alone (e.g., methanogenic propionate degradation). Because these reactions operate at extremely low energetic yield,

even small changes in electron carrier concentrations can strongly shift reaction energetics and feasibility. Previous studies have primarily focused on the metabolic pathways responsible for producing and consuming electron carriers within cells. In contrast, the physical transport processes governing how these carriers move between cells, and how transport limitations influence cellular metabolism, remain poorly understood.

Here, we investigated how fluid flow, induced by different mixing intensities, alters metabolic activity and gene expression in a Pelotomaculum–Methanospirillum coculture that syntrophically converts propionate to methane. First, a thin-film–based mass transfer model was developed to estimate near-cell concentrations of hydrogen and formate, representing a more accurate estimation to cellular microenvironment instead of commonly used bulk measurements. Next, these transport-constrained concentrations were used to calculate the thermodynamics of individual redox reactions during syntrophic propionate degradation. Metatranscriptomic analysis was performed to identify genetic responses, with a focus on electron transfer pathways.We found that increased fluid flow reduces near-cell electron carrier concentrations and upregulates key genes involved in electron transfer, including cytoplasmic bifurcating hydrogenases, membrane-bound hydrogenases, formate dehydrogenases and transporters. By integrating transport modeling, thermodynamic analysis, and metatranscriptomics, this study reveals a previously underappreciated role of mass transfer in shaping microbial metabolism under thermodynamically constrained conditions.

Long-term nitrogen enrichment alters spatial turnover and the relative impact of selective agents in soil bacterial communities

Isabelle Lakis, Caroline Oldstone-Jackson, Sierra L Bedwell, Katy D Heath, Anthony C Yannarell

Department of Microbiology, University of Illinois Urbana-Champaign

The processes structuring microbial communities are broadly categorized as selection, drift, speciation, and dispersal (Velland 2010). Together, these processes generate stochastic and deterministic patterns in community assembly. Many studies evaluate environmental drivers or spatial distance in isolation rather than testing them together. Here, we examine the combined effects of selection and dispersal on soil microbial communities.

We leveraged replicated long-term nitrogen (N) enrichment and unfertilized control plots at the NSF W.K. Kellogg Biological Station Long-Term Ecological Research site (KBS LTER) to test how nutrient enrichment reshapes microbial responses to spatial distance, soil chemistry, and plant communities. We characterized bacterial composition using full-length 16S amplicon sequencing and quantified dissimilarity among samples spanning ~1–800 m within each treatment. We fit distance–decay

models and used multivariate approaches to partition the contributions of N treatment, spatial distance, soil pH, and plant community composition.

Long-term nitrogen addition strongly reorganized soil bacterial communities, producing clear treatment-level clustering and the greatest dissimilarity between N-enriched and control soils. In control plots, variation in community composition was consistently explained by plant community composition and soil pH, indicating structured and predictable assembly. The strong correspondence between plant community and spatial distance suggests environmental selection rather than dispersal limitation.

In contrast, microbial communities in nitrogen-enriched plots were less explained by these factors. Although plant community composition remained the strongest correlate, its explanatory power was reduced, and the effects of spatial distance and soil pH were weakened. Overall, nitrogen enrichment reduced the extent to which community variation could be explained, suggesting the microbial community diversity is relatively decoupled from spatial structure and key environmental drivers. Together, these results indicate that chronic nutrient enrichment not only shifts microbial community composition but also reweights the relative importance of selective agents in soil community assembly.

Grain prebiotic content shapes individual gastrointestinal motility and postprandial metabolic responses

[1]Benjamin A. Levine, [1]Jordan E. Rindels, [1,2]Brett R. Loman

[1]Division of Nutritional Sciences, [2]Department of Animal Sciences University of Illinois Urbana-Champaign

Genetic and non-genetic factors, such as enteric microbiome composition, diet, sex, age, and physical activity, contribute to interindividual variability in postprandial responses to identical meals. Gastrointestinal motility (GIM) is the physiologic process coupling the digestion and absorption of food and its nutrients to the postprandial appearance of those nutrients in circulation. Thus, we hypothesize that individual GIM responses may be a critical determinant of postprandial metabolism, and will help explain some of the remaining interindividual variability. This human randomized, controlled, balanced, crossover trial determined the extent to which GIM responses to acute whole grain (WG, prebiotic rich) & refined grain (RG, prebiotic poor) bread ingestion mediate postprandial metabolism.

Thirty-four participants (17 males, 17 females; aged 20-59) underwent acute feeding of 4.3 oz of WG (12g fiber) and RG (3g fiber) rye bread, each served with 0.7 oz of butter and ad libitum water, with a one-week washout between bread arms. Participants completed a 12-hour fast before each arm with a one-week washout between arms. GIM metrics were measured via SmartPill motility capsules in

tandem with blood metabolites (glycemic and lipemic) measured by capillary blood analyzers. Blood metabolites were assessed across 10 postprandial timepoints (Baseline, 0.5,1,1.5,2,3,4,5,6,8 hours), and softly clustered post-hoc via via mFuzz algorithm. Participants broke their fast after blood sampling, and stool was collected upon defecation of the Smartpill.

Females tended to have longer gastric emptying ($p = 0.06$), less frequent antral contractions ($p = 0.05$), and later and broader blood glucose excursions than males. WG tended to reduce small intestinal contractions ($p = 0.07$) without affecting transit time or pressure (both $p > 0.05$). WG prolonged colonic transit in females but not males (Sex $p = 0.0089$, Bread type $p = 0.58$, Sex $p =$

0.04), which was paired by females having less frequent colonic contractions vs males (Sex $p = 0.02$). Colonic contraction frequency were moderately correlated with transit time with the WG arm ($R^2 = 0.48$), but not the RG conditions ($R^2 = 0.003$).

Anthropogenic and environmental factors affecting virus communities associated with honeybee-Varroa system.

Valeria Grande[1,2,3], Cristina Marzachì[1,2], Simone Tosi[1,2,3], Valeria Trivellone[1]

[1]Prairie Research Institute, University of Illinois at Urbana-Champaign
[2]Department of Agricultural, Forest and Food Science, University of Turin
[3]Institute for Plant Protection, National Research Council, Turin

Honeybees (*Apis mellifera*) are among the most economically and ecologically important pollinators, yet colonies continue to experience significant losses worldwide due to interacting stressors, including anthropogenic pressure, environmental change and biological associates. Among these, the tripartite interaction between honeybees, *Varroa destructor*, and associated viruses is a major driver of colony mortality. While viruses are ubiquitous in managed populations, their dynamics are influenced by management practices and environmental context. However, large-scale, empirical studies examining the co-occurrence of these associations across environmental and anthropogenic gradients remain limited. This study investigates how environmental context (landscape and climate), management practices (e.g. acaricide use) and biological interactions shape honeybee population dynamics, *Varroa* infestation, and virus composition. Data were integrated from two independent projects (2017-2023), comprising over 260 colonies across 30 locations in Italy and France. Colonies were screened for common honeybee viruses using RT-qPCR. *Varroa* infestation was quantified using the detergent wash method, and colony health (adult bees and brood) was estimated using the Coleval method. Climatic variables were obtained from CHELSA, and landscape metrics were derived from the CORINE Land-Cover

2018. Variable selection was performed using Pearson correlation and forward selection. Variation partitioning was then applied to quantify the relative contribution of each set of explanatory variables to each component of the tripartite association. Climate and management practices emerged as the primary drivers across the system. Climate explained 16.3, 6.4% and 2.8% of the variance in brood abundance, *Varroa* and virus community, respectively; while management accounted for 5.0, 2.1 and 3.9% Focusing on the virus community, these results indicate that both climatic and beekeeping practices play a central role in shaping viral dynamics, although a substantial proportion of variance remains unexplained. Ongoing analyses are evaluating the potential mediated effects of honeybee health on virus community structure to better account for unexplained variability.

Microbial delivery of antibodies to combat porcine respiratory disease complex

Mitchell L. Bryant[1], Mark L. Tarabey[1], Shannon J. Sirk[1].

[1] Department of Bioengineering, University of Illinois Urbana-Champaign

Porcine respiratory disease complex (PRDC) is responsible for billions of dollars in loss to the meat industry every year. PRDC results from interactions between host microbiota, environmental stress, and pathogens. Current treatment for PRDC relies on antibiotics, which leads to antimicrobial resistance and does not address viral disease. Viral disease in livestock is often not addressed due to the prohibitive cost of antibody therapy. To develop a cost effective non-antibiotic alternative response to PRDC, we aim to engineer members of the host microbiota to secrete neutralizing antibodies against viral pathogens implicated in disease. Specifically, we engineer lactic acid bacteria present in the porcine respiratory tract to secrete antibody fragments targeting influenza and porcine respiratory syncytial virus, two major viruses implicated in PRDC. To achieve this, we use a modular synthetic biology platform to engineer species of pig respiratory lactic acid bacteria to secrete high levels of antibody fragments into the respiratory tract. These antibodies are assessed for viral neutralizing capacity by their ability to coat viral particles and prevent entry into host cells. After in vitro validation of the platform, these engineered strains are returned to the host respiratory niche to provide antibiotic-free protection against viral pathogens that drive PRDC. By delivering antibodies with commensal bacteria, we can offer a wider range of protection against viral pathogens and reduce the cost of antibody therapy as to be economically viable for use in livestock. This treatment has the potential to improve livestock health and reduce the spread of viral disease in agricultural settings.

Microplastics and global change stressors as drivers of pathogen ecology and antimicrobial resistance in soil-food systems

Jayita De, Pratik Banerjee

Department of Food Science and Human Nutrition, University of Illinois, Urbana-Champaign

Microplastics (MPs) and nanoplastics (NPs) are emerging contaminants widely detected in agricultural soils due to plastic use, wastewater irrigation, and the use of biosolids as amendments. Beyond their persistence, MPs can act as vectors for co-contaminants such as heavy metals and anthropogenic organic and inorganic pollutants, creating plastisphere microenvironments that may influence microbial communities, foodborne pathogen behavior, and antimicrobial resistance (AMR). However, the mechanistic role of MPs and co-occurring global change stressors (GCSs) in shaping pathogen ecology and resistance dynamics remains poorly understood.

This study integrates mechanistic and environmentally relevant approaches to evaluate how MPs, contaminant interactions, and climate-linked stressors influence the physiology of *Salmonella enterica* and soil microbiomes. In a current study, we investigate how plastic weathering and lead (Pb) adsorption onto low-density polyethylene (LDPE) MPs alter surface reactivity and promote biofilm formation, persistence, and gene expression in *S. enterica*. In our previous study, it was demonstrated that polystyrene (PS) NPs induce oxidative stress, membrane damage, enhanced biofilm formation, and transient activation of virulence and AMR-associated genes, indicating adaptive trade-offs under stress. We hypothesize that aged, Pb-loaded MPs will further amplify these responses and act as hotspots for resistance enrichment.

In parallel, controlled soil microcosm experiments are used to evaluate temperature-dependent effects of key GCSs, including MPs, PFAS, heavy metals, glyphosate, and biological amendments, on microbial community structure and ARG mobilization. Using *16S rRNA* sequencing, metagenomics, and network-based analyses, we assess shifts in microbiomes, resistomes, and the potential for horizontal gene transfer (HGT) under ambient and elevated temperatures.

Essentially, this work advances a One Health framework by linking environmental contamination to food safety risks, demonstrating how plastics and climate-driven stressors can reshape pathogen behavior and promote AMR dissemination across the soil-food-human continuum.

Talk consistency of community composition of Wolbachia in Penenirmus sp. feather lice (Psocodea: Ischnocera)

Ember B. Clodfelter, Matthew Doremus, Kevin P. Johnson

Department of Entomology, University of Illinois at Urbana-Champaign

Endosymbiotic bacteria are common in insects with nutritionally limited diets. These bacteria often provide necessary nutrients for their host, resulting in host-symbiont interdependence and long-term coevolutionary relationships. Parasitic lice, which have a diet rich in amino acids but lacking key vitamins, host a wide diversity of endosymbiotic *Wolbachia*. Some strains of *Wolbachia* are potential nutritional mutualists while others are likely reproductive parasites. The feather? louse genus *Penenirmus* hosts *Wolbachia* strains representing three supergroups variably associated with either reproductive manipulation or nutrient supplementation. Initial evidence shows that some strains have long-term associations with their hosts, a pattern suggestive of a stable cophylogenetic evolutionary relationship. Other strains do not show cophylogenetic signals, suggesting more recent symbiont acquisition. This study proposes sequencing additional lice from this genus and comparing through genome-resolved metagenomic techniques the consistency of their bacterial endosymbiont communities.

Harnessing polyphosphate-accumulating microbes to mitigate phosphorus losses from Illinois agroecosystems

Aislinn Geedey (Graduate Student)[1], Connor Sible[2], Fred Below[2], Angela Kent[1]

[1]Department of Natural Resources and Environmental Sciences, University of Illinois Urbana-Champaign
[2]Department of Crop Sciences, University of Illinois Urbana-Champaign

Phosphorus (P) runoff is typically associated with soil erosion and surface runoff, but recent research has demonstrated that subsurface drainage tiles can be significant conduits for P losses from relatively flat Illinois agroecosystems. Closed depressions within fields often collect surface runoff P and water, leading to waterlogged soils that leach P into tile drainage systems, increasing P losses. The unique metabolism of polyphosphateaccumulating organisms (PAOs) presents an opportunity for novel P retention methods in soils. Under anoxic (oxygen-depleted, high nitrate) conditions, microbial PAOs take up P from the environment and store it as polyphosphate (PolyP). Stored P can later be released as orthophosphate by cleaving stored PolyP molecules, allowing for plant uptake of P.

This project aims to evaluate and determine how experimental management treatments (nitrogen fertilization and carbon application) implemented in a field experiment under rainfed and flooded conditions associated with P loss may influence P dynamics and nutrient losses via changes in microbial PolyP cycling. These management strategies are based on existing knowledge of PAOs in wastewater systems, and are designed to stimulate microbial PolyP accumulation to reduce P losses via tile drainage systems. By comparing measures of microbial PolyP cycling, soil P leaching losses, and plant P uptake in our field experiment, we

can better understand how management strategies may influence microbial P cycling and nutrient losses.

This presentation will discuss the preliminary results of two field seasons of data, including qPCR quantification of key PolyP-cycling genes, DNA sequencing results evaluating presence and relative abundance of soil PAO taxa, and the impact of our experimental management strategies on crop yield, crop P uptake, and P losses from soils.

Carbohydrates, cavities, and collective dynamics: How dental microbes evolve to environmental stressors upon exposure to sugar alternatives

Shay, M.R., Wood K.B., Tenuta L.M.A, Stockbridge R.B., Lin, N., Zaman, L.

Cellular & Molecular Biology, Chemical Engineering, Ecology & Evolutionary Biology, University of Michigan

Dental cavities are primarily a polymicrobial disease, wherein cariogenic microbes (such as *S.mutans*) consume fermentable sugars and create highly acidic conditions that select against commensal microbiota (such as *S.gordonii*). Some commonly used sugar alternatives, such as xylitol and erythritol, are non-fermentable and known to inhibit the growth of cariogenic microbiota. Historically, fluoride has been considered the most effective agent to control dental caries and is very beneficial with the primary effect considered the remineralization of the enamel, though it can also act as a potent antimicrobial in certain conditions. Increasingly, toothpastes with high concentrations of both fluoride and non-fermentable sugar alternatives are being prescribed to cavity-prone patients. It is unknown how chronic exposure to sugar alternatives impacts the adaptation of cariogenic and non-cariogenic oral microbes to chronic fluoride stress.

For this work, we evolved *S.mutans* and *S.gordonii* in different carbon environments (glucose, sorbitol, xylitol, and erythritol) either in the presence of fluoride to identify the impact of sugar alternatives on the modes and effectiveness of fluoride resistance. We observed species-specific differences in how effectively the populations were able to adapt to fluoride challenge in each carbon environment and identified variability in mutation frequency and mechanisms of resistance using whole genome sequencing. A notable instance of parallel evolution occurred in *S.mutans* populations exposed to fluoride and sugar alternatives (sorbitol, erythritol, or xylitol), wherein a previously unreported mutation mimicking lysine acetylation occurred in the only nucleotide associated protein (HU) in *S.mutans*. When the ancestor background was transformed with only this point mutation, it resulted in robust changes to fluoride resistance. RNA-sequencing confirmed that the point mutation results in drastic shifts in gene expression compared to the ancestor when grown in low level fluoride.

Isolation and characterization of microbiota from human pancreatic tumors and duodenum

Awad, D., Attebury, H., Hong, R., Kim, K., Zhang, L., Bischoff, A., Achi, S., denDekker, A., Lesniak, N., The, S., Nieto Carrion, J. A., Nelson, N. S., Strayhorn, C., Griffith, B. D., Watkoske, H. R., Peterson, N., Lenard, M., Muir, A., Sahai, V., Li, G., Frankel, T., Pasca di Magliano, M., Lyssiotis, C. A., Schmidt, T. M., & Daley, D.

Department of Molecular and Integrative Physiology, University of Michigan

Pancreatic ductal adenocarcinoma (PDAC) has a unique tumor microbiome, and the depletion of gut bacteria or fungi using oral antibiotic/antifungal cocktails leads to a decrease in pancreatic tumor burden in mice. However, functional studies deciphering the mechanisms via which the microbiome influences tumor growth remain rare due to the limited availability of clinically relevant microbiota.

In order to study the gut and tumor microbiome of pancreatic cancer patients in more detail, we isolated bacteria and fungi from the duodenum and tumor of pancreatic cancer patients undergoing surgical resection, using six optimized media compositions under hypoxic and anoxic growth conditions.

The most dominant bacteria isolated from pancreatic tumor was a *Klebsiella oxytoca* strain (UMKO1), which we characterized to provide insight on the potential impact of individual microbes on the tumor microenvironment. We performed whole genome sequencing and untargeted/targeted metabolomics (LC-MS/MS) on UMKO1 cultured in specific pancreatic tumor nutrient conditions. UMKO1 produces several key indoles, such as indole-3-acetic acid, which are known to influence tumor immunity and chemotherapy response in pancreatic cancer patients. UMKO1 also possesses a gene for the long form of cytidine deaminase, which catabolizes the chemotherapeutic agent Gemcitabine, rendering it ineffective. Using an ex-vivo transplant system, we assessed the impact of UMKO1 in the pancreatic tumor microenvironment. Bacterial products/metabolites in the supernatant of UMKO1 inhibited granzyme B production from CD8 T cells, which was not observed with other isolated Klebsiella species. This highlights the unique effects of UMKO1 on the pancreatic tumor microenvironment.

Taken together, the isolation of these clinically relevant microbiota will allow us to intricately study the metabolic mechanisms via which the tumor microbiome impacts pancreatic cancer progression and response to therapy.

From cortisol to cancer-promoting signals: B. desmolans DesAB shapes gut epithelial responses.

Freiberg, M., Abernathy M., Fernandez-Materan F.V., Ridlon, J.M.

Department of Animal Sciences, University of Illinois Urbana Champaign.

The ability of gut bacteria to convert C_{21} glucocorticoids into 11-oxygenated C_{19} androgens has been known for almost half a century. DesA and desB genes encode steroid-17,20-desmolans that can side-chain cleave cortisol to produce 11β-hydroxyandrostenedione (11β-OHAD). These genes were first identified in Clostridium scindens ATCC 35704, within a cortisol induced operon (desABCD). DesAB genes, along with steroid-17,20-desmolans activity were also identified in Butyricoccus desmolans ATCC 43058. Although 11-oxygenated C_{19} androgens, such as 11β-OHAD are considered major contributors to the development of castration-resistant prostate cancer, and DesAD can metabolize other endogenous and exogenous glucocorticoids, B.desmolans DesAB has never been characterized, and the effects of 11β-OHAD on gut function remain unexplored.

We cloned, overexpressed, and purified B.desmolans rDesAB and evaluated its enzyme kinetics and substrate specificity using a coupled, continuous spectrophotometric assay and LC-MS. Cortisol was the preferred substrate (Km, 19.7±1.47 μM; Vmax, 1.52±0.16 μmol·min^{-1}), while 11deoxycortisol and cortisone were also metabolized with 52% and 11% relative activity, respectively. To assess host effects, primary mouse intestinal epithelial cells were treated with 11β-OHAD, cortisol or vehicle control for six hours. RNA sequencing revealed upregulation of proinflammatory and carcinogenic pathways, along with inhibition of cell cycle and DNA replication pathways. Additionally, ELISA of secreted cytokines showed that 11β-OHAD-treated cells, but not cortisol or control treated cells, had higher VCAM1 and CSF1 levels, while IGFBP6 levels were significantly lower. Western-blot analysis confirmed CSF1 receptor (CSF1R) levels were also elevated. Taken together, our data indicate that B.desmolans production of 11β-OHAD leads to proinflammatory, cancer-promoting conditions in the host gut. This is the first study to characterize B.desmolans DesAB and its product effect on host physiology, highlighting the potential of gut microbiota-derived steroid metabolites to modulate host intestinal physiology and health.

Characterization of methylotrophic bacteria from retting hemp

Kayla M. Sharkey, Luke A. Moe

[1]Department of Plant and Soil Sciences, University of Kentucky, KY 40506

Retting hemp is a crucial process for preparing hemp fibers for use in paper, industrial textiles, and building materials. Field retting is the process where hemp stalks are cut and laid in the field for several days where microbes degrade hemicelluloses and pectin, making it possible to separate the bast fibers from the hurd core which is necessary prior to further processing. The understanding of the microbiome of hemp during the retting process is still not fully understood, and

limits improvement of hemp fiber yields using synthetic communities during the retting process.

Our previous study analyzing the metagenomes of hemp during retting illuminated that methylotrophic bacteria appear to play a key role in the initial degradation of pectin during the hemp retting process. To investigate this a library of methylotrophs was developed for further study. Methylotrophic bacteria were isolated from field retted hemp using a wash solution in a bag mixer, serially diluted, and plated on M9 minimal media with methanol as the carbon source. Isolates' 16S rRNA genes were then sequenced for taxonomic classification. Isolates capability to degrade pectin will be assessed using DNS assay. Forty-four isolates have been sequenced and have been classified as ten species in the genera *Methylorubrum* and *Methylobacterium*. Further work is ongoing. This study's findings will help guide future synthetic community studies for improving retting efficiency.

Local and global comparison of Mobile Genetic Elements reveal gene carriage associated with incompatibility groups

Isaiah Goertz, Edward Andrews, Jim Lowe, Rebecca Smith, Rachel Whitaker

School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, IL

Mobile genetic elements (MGEs) are key factors in the spread of antibiotic resistance genes (ARGs) and other key genes of interest. MGEs are extrachromosomal elements that can live in hosts either by circularizing in the cell or inserting into the host chromosome. One of the types of MGEs well known for ARG dissemination are plasmids. Plasmids are extrachromosomal elements that can circularize or integrate into the host chromosome. They can spread ARGs into new hosts and disseminate through a community. One of the main ways plasmids are classified is by incompatibility groups (Inc group), where plasmids with the same incompatibility group cannot be stably inherited due to shared replication machinery. This is a significant factor in how plasmids compete and interact, as plasmids of the same Inc group will be lost overtime due to competition. However, plasmids can also coexist with other plasmids of different Inc groups, allowing for gene exchange and recombination events to occur. This gene carriage and frequent change of genetic material can lead to MGEs becoming mosaics from gene insertions and recombination events, leading to possible new ARGs. The dissemination of ARGs is of major focus in One Health, where the health of the human environments and livestock environments impact each other. In our pilot study, we investigated 28 E. coli isolates sampled from a co-housed swine population of nine pigs. Investigating plasmid diversity, we identified an isolate possessing five MGEs with different Inc group designation. When comparing the MGE sequences to the local isolates and to the NCBI nucleotide database we see

different patterns of gene carriage, with some genes co-occurring more frequently than others. However, we saw that the genes that did co-occur were found in the global database on elements within the same Inc group as their host MGE.

Linking niche diversity to phylogenetic relatedness in freshwater bacteria assemblies

Kissel, S. H. & Hartmann, E. M.

Department of Biology, Northwestern University

Aquatic bacteria aid in the cycling of nutrients and removal of pollutants in freshwater ecosystems and are critical to lake stability. Current methods of taxonomic classification rely on core functional genes to identify bacteria. However, the resulting observed changes in abundance and diversity of taxa mask phenomena like horizontal gene transfer, changes in gene copy number, and overall gene family presence. To better understand the relationship between traditional taxonomy, functional categorization, and niche occupancy, we use two decades of publicly available metagenome assembled genome (MAG) data from Lake Mendota in Madison, WI. Through a combinatorial approach of pangenome analysis and Topological Data Analysis (TDA), we look at the presence and absence of gene families in 10 large clusters of MAGs sharing Average Nucleotide Identity (ANI) of 98.5%. We compare the clusters based on two main distinctions: class delineation (Cyanobacteria, Bacteroidia, Planctomycetia, Alphaproteobacteria) and plasticity (open or closed pangenome). Preliminary findings indicate that clusters fall on a continuum of plasticity with a strongly conserved core gene section. Most interestingly, specific motility and phage related genes, which are expected to correspond to horizontal gene transfer, show differential presence patterns across open and closed pangenome clusters. TDA further indicates complicated dynamics in bacteria diversity through the presence of 1-dimensional holes in the data, possibly suggesting new interpretations of phylogenetic relatedness. Together, these results demonstrate that genetically identified clusters have different patterns in functional adaptability indicating a need for revision in the current taxonomy model. Future efforts will be needed to validate these data across a broader array of environments and with more accurate sequencing techniques.

Linking belowground mutualisms to aboveground growth trends associated with the invasive legume Lespedeza cuneata and native legumes in a restored prairie habitat

Mac Johnson and Anthony Yannarell

Department of Natural Resources and Environmental Sciences, University of Illinois Urbana-Champaign

Invasive plants can generate legacy effects where soils are conditioned to promote the invader or suppress native species by influencing soil microbial communities. *Lespedeza cuneata* , a species of concern to North American prairies, is an invasive legume that rapidly proliferates in disturbed sites and can outcompete native plants. To determine how soil invasion history affects the growth and nodule communities of *L. cuneata* compared to native prairie legumes, I conducted a greenhouse experiment where I inoculated four legume species – *Lespedeza cuneata, Lespedeza virginica, Dalea purpurea, Desmodium illinoense* – with live soil from either highly invaded or uninvaded areas of a restored prairie. The species with the lowest mortality were *D. illinoense* (30%) and *L. cuneata* (33%) compared to *D. purpurea* (54%) and *L. virginica* (93%). Although *D. illinoense* and *L. cuneata* exhibited similar survival, *D. illinoense* individuals were generally larger and healthier than *L. cuneata* survivors. Biomass accumulation and nodule count were highest in *D. illinoense* with no significant effect of treatment; however, nodule count for *L. cuneata* increased in invaded soil versus uninvaded soil. Both rhizobia and non-rhizobia were isolated from the nodules of *D. illinoense* and *L. cuneata* , but there were many more non-rhizobia found in *Desmodium* nodules. The differences in biomass accumulation, general plant health, and nodule endophyte diversity between *D. illinoense* and *L. cuneata* may indicate unique growth strategies during early life stages. The diverse strain library (n = 172) generated from this experiment will also serve as a future resource for investigating how rhizobia and non-rhizobia associate with and benefit native legumes compared to *L. cuneata* , either through co-inoculation experiments or strain-based enzymatic assays. These results reinforce previous findings that *L. cuneata* alters soil conditions to promote its own growth during invasion but raise intriguing questions about the role of life history traits in *L. cuneata* invasions.

Characterizing the microbiome and prevalence of Wolbachia in Culex pipiens complex and Culex restuans mosquitoes in the Midwest United States

Cloud, R. E., Irwin, P., Muturi, E. J., & Cáceres, C. E.

Program in Ecology, Evolution and Conservation Biology, University of Illinois Urbana-Champaign

Despite the ecological importance of symbiotic relationships, few studies have explored how microbial communities vary across species complexes or within hybrid zones. We characterized the spatial and temporal variation in microbial communities in *Culex pipiens* complex and *Culex restuans* mosquitoes, the most important vectors for West Nile virus in the midwestern United States. *Cx. pipiens* complex and *Cx. restuans* mosquitoes were collected monthly from May to September 2023 from three geographic regions (Champaign County, IL, Cook County, IL and Dane County, WI). DNA from individual mosquitoes and their bacterial communities were sequenced using Illumina NovaSeq amplicon

sequencing. Microbial composition differed between *Cx. pipiens* complex and *Cx. restuans,* driven by high relative abundance of *Wolbachia* in *Cx. pipiens* complex compared to *Cx. restuans,* which also contributed to greater within-species microbiome variability. However, despite limited sample sizes for some forms and hybrids, there were no detectable differences in the bacterial diversity or community composition among the five observed *Cx. pipiens* forms. Microbial diversity also varied regionally and over the sampling season, increasing later in the season. These findings demonstrate a microbial convergence within *Cx. pipiens* complex and reveal that spatiotemporal factors influence acquisition of environmentally acquired microbes highlighting the dynamic nature of mosquito-microbe interactions.

Targeted fiber fermentation for inflammatory and metabolic disease intervention

Ziyu Zhou[1,8], Ka Lam Nguyen[1,8], Sijie Chen[1,8], Yuexi Wang[1], Min Li[1], David M. Bianchi[2], Weihao Ge[2], Shanny Hsuan Kuo [3,10], Salwa Gharieb[1], Emily Tung[1], Ronak Parmar[1], Jie Ji[1], Rohit Khorana[1], Ahmed Hetta[4,5], Isaac Cann[4,5], Roderick I. Mackie[4,5], Gee W. Lau[3] , Jing Yang[1], Wenyan Mei[1,5,6,7]

1. Department of Comparative Biosciences, University of Illinois Urbana-Champaign, Urbana, Illinois
2. National Center for Supercomputing Applications, University of Illinois Urbana-Champaign, Urbana, Illinois
3. Department of Pathobiology, University of Illinois Urbana-Champaign, Urbana, Illinois
4. Department of Animal Sciences, University of Illinois Urbana-Champaign, Urbana, Illinois
5. Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, Illinois
6. Division of Nutritional Sciences, University of Illinois Urbana-Champaign, Urbana, Illinois
7. Cancer Center at Illinois, University of Illinois Urbana-Champaign, Urbana, Illinois
8. These authors contributed equally to the work.

Consuming a Western diet, characterized by high-fat, high-sugar, and low-fiber foods, has been linked to compromised gut microbiota function and the rising metabolic and inflammatory disorders that affect millions of people worldwide, causing enormous human suffering and economic burden. In contrast, a higher intake of dietary fiber is associated with a more diverse gut microbiome and a reduced risk of these diseases. The health benefits of fermentable fibers depend on fiber-utilizing gut microbes that convert complex polysaccharides into bioactive metabolites with protective effects. Yet, the fiber-deficient Western diet has reduced or eliminated many fiber-fermenting taxa from the gut microbiota,

diminishing the benefits of dietary fiber for many individuals. To address this gap, we aim to develop synergistic synbiotics that pair fiber-fermenting microbes with their preferred substrates as therapeutic dietary supplements. We recently made an exciting finding: the human gut commensal *Bacteroides intestinalis (B. intestinalis)* ferments insoluble wheat arabinoxylan (inWAX) *in vivo*, a complex polysaccharide abundant in dietary fiber, leading to enhanced host resistance to inflammatory bowel diseases and improved glucose tolerance in high–fat diet–induced obesity. Mechanistically, the intervention selectively increases the hepatic and microbial production of anti-diabetic and anti-steatotic bile acid species and promotes the microbial transformation of anti-inflammatory and antioxidant phenolic compounds. This metabolic shift is accompanied by coordinated transcriptomic reprogramming in the colon and spleen, activating gene networks that support triglyceride metabolism, circadian regulation, and immune protection. Our findings identify *B. intestinalis* as a key driver of dietary fiber-driven metabolic and immune benefits, and underscore the therapeutic potential of the *B. intestinalis*–inWAX synbiotic for mitigating inflammatory and metabolic diseases.

From storage to fermentation: Gut microbiota preservation shapes microbial communities and metabolic outputs in vitro

Guzmán Sánchez, M., Jackson, P., Adams, S., Lindemann, S.

Food Science Department, Nutrition Science Department, Purdue University

In vitro gut fermentations are often used to assess microbial metabolism and ecology. These systems are usually inoculated using fresh feces; however, logistical limitations often demand sample storage. However, no consensus exists on optimal preservation methods or their impact on fermentation reliability; a systematic evaluation of storage effects is needed. This study aimed to evaluate the effects of multiple short-term fecal storage methods on metabolic output and community structure for *in vitro* fermentations.

Fecal samples from four healthy adults were used. Each was divided into 11 aliquots, including a fresh control and five storage conditions: snap freezing in liquid nitrogen before transfer to –80°C, direct storage at –80°C and at –20°C, storage over blue ice packs (~4°C) with and without 10% w/v Oxyrase. Each was assessed for 24 and 48 h. Aliquots were subjected to a seven-day sequential batch fermentation with inulin (IQ) and sorghum arabinoxylan (SAX) separately. Gas production, pH, and short-chain fatty acids (SCFA) were measured at the end of each 24 h fermentation. Fecal input, batch, and enrichment communities (day 1 and 7) were assessed by 16S rRNA sequencing.

Batch fermentations from stored samples had greater variability and deviated from fresh controls, including altered pH and SCFA production, particularly reduced propionate, in both SAX and IQ. Storage significantly altered baseline and batch community composition within donors in both substrates (PERMANOVA, $p < 0.01$). Community structure changes were donor-specific, with decreases in *Bacteroides* spp. most noticeable. Sequential passaging mitigated these metabolic and compositional effects; outputs converged toward fresh controls, storage-driven clustering was attenuated, and most OTUs significantly altered in batch communities showed no significant fold changes in enrichment communities. These findings suggest that sample storage can cause stochastic shifts in batch fermentations, compromising reliability. However, sequential passaging can be used to derive consistent microbial lineages for further analyses.

The germ-free zebrafish model: Long-Term viability through gamma-irradiated diets

Lydia Okyere[1], Angela Di Fulvio[2], Christopher Gaulke[1,3,4,5,6,7]

[1]Department of Pathobiology, University of Illinois Urbana-Champaign
[2]Department of Nuclear, Plasma, and Radiological Engineering, University of Illinois Urbana-Champaign
[3]Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign
[4]Personalized Nutrition Initiative, University of Illinois Urbana-Champaign
[5]Cancer Center at Illinois, University of Illinois Urbana-Champaign
[6]National Center for Supercomputing Applications, University of Illinois Urbana-Champaign
[7]Interdisciplinary Health Sciences Institute, University of Illinois Urbana-Champaign

Host-associated microbiota regulate key host processes, including nutrient uptake, immunity, and xenobiotic metabolism and their disruption is linked to disease though causal mechanisms mostly remain unclear. Zebrafish gnotobiotic models provide a scalable, cost-effective system for studying causal host-microbiota interactions. However, current husbandry methods relying on live or autoclaved diets restrict their use to early developmental stages, limiting the study of mature host-microbiota relationships. In this study, we evaluated a simple approach for long-term maintenance of gnotobiotic zebrafish using gamma-irradiated chow diets and assessed impacts on host growth, gene expression, and microbial community composition. We developed and evaluated a protocol for long-term husbandry of gnotobiotic zebrafish using sterile gamma-irradiated chow diets. Conventional zebrafish were first fed diets irradiated at multiple doses to determine effects on host growth, survival, or gut microbial communities. Microbial composition was characterized using 16S rRNA gene sequencing. The protocol was then used to maintain germ-free zebrafish for extended developmental periods,

and host transcriptomic responses were evaluated using RNA-Seq. Gamma-irradiated diets did not significantly affect growth or survival in conventional fish and produced only modest shifts in microbiome composition. In contrast, germ-free zebrafish maintained on irradiated diets for 55 days post-fertilization exhibited reduced body size compared with colonized controls. Transcriptomic analyses revealed alterations in host gene expression, with enrichment of pathways associated with immune function, xenobiotic metabolism, development, liver function, and lipid metabolism. Notably, several host transcriptional patterns correlated with the abundance of specific microbial taxa in colonized animals. These results highlight the role of microbial colonization in regulating host growth and metabolic development and importantly demonstrate that gamma-irradiated diets enable germ-free zebrafish husbandry beyond early larval stages. This study expands the utility of this model for future work that can leverage this system to define mechanisms of interactions between host and microbiota across lifespan.

---

microbial eukaryotic activity on marine sinking particles: Metabolic niche partitioning and links to POC flux

Isha Kalra[1], Brittany P. Stewart[1], Jennifer L. Beatty[1], Samantha J. Gleich[1], Fuyan Li[2], Eric Grabowski[2], David M. Karl[2], Edward F. DeLong[2], and David A. Caron[1]

[1] Marine and Environmental Biology Division, University of Southern California, Los Angeles, CA
[2] Department of Oceanography, University of Hawai'i at Mānoa, Honolulu, HI

Sinking particles are a central component of the marine biological pump, transporting fixed organic carbon from surface waters to the deep ocean. These particles consist of sinking phytoplankton cells, aggregates, and fecal pellets, and are sites of intense microbial transformation. While bacterial degradation is well-studied, the functional roles of particle-associated microbial eukaryotes (protists) remain poorly resolved. We investigated the diversity and metabolic activity of protistan communities near Station ALOHA (North Pacific Subtropical Gyre) following a summer diatom bloom, using two complementary 24-hour sediment trap approaches: Daily Particle Interceptor Traps (PITs) and NetTraps. Metatranscriptomic samples were collected from Daily PIT deployments at 150 m and NetTraps deployed across multiple depths (150 - 300 m), alongside surrounding water column, enabling resolution of both temporal and vertical patterns in protistan activity.

Our results reveal distinct metabolic niche partitioning between particle-associated and free-living communities at the base of the euphotic zone (150 m). Differential expression analysis showed that sinking particles were significantly enriched in transcripts related to phagotrophy, while the surrounding water column was dominated by phototrophic activity. This functional divergence was further

established by a significantly lower phototrophy-to-heterotrophy ratio (P:H) on particles compared to water column. Furthermore, temporal shifts in protistan activity at this depth were tightly coupled to particulate organic carbon (POC) flux. We identified several phagotrophy and cell adhesion related transcripts, including vinculin, thrombospondin 1, and Arp2/3 complex, that were strongly positively correlated with POC flux. Additionally, specific particle-enriched taxa such as labyrinthulid *Aplanochytrium*, exhibited transcriptional activity that significantly and positively correlated with POC flux. These findings suggest that sinking particles serve as hot spots for eukaryotic scavenging and predation, and that particle-associated protists actively contribute to metabolic transformation of sinking organic matter during episodic export events in the oligotrophic ocean.

Viral exploitation of bacterial dormancy: Entrapment, persistence, and reactivation in spore-forming microbes.

Sungyun Kang, Joy O'Brien, Jay Lennon
Department of Biology, Indiana University

Microorganisms frequently encounter environmental conditions that limit growth and survival. One common strategy to persist under these conditions is dormancy, a reversible state in which cellular activity is greatly reduced. Dormancy has traditionally been viewed as a response to abiotic stress, such as nutrient limitation or antibiotic exposure. However, growing evidence suggests that dormancy also plays an important role in shaping interactions between microbes and their viruses (phages). While dormancy can reduce susceptibility to infection, some viruses have evolved strategies to exploit this state.

This work explores how viruses interact with the dormancy program of spore-forming bacteria, using Bacillus subtilis as a model system. Certain viruses can co-opt the bacterial sporulation process using genes acquired from their hosts, allowing viral genomes to become trapped within developing spores. These "virospores" protect viral DNA from environmental stress and enable long-term persistence outside of actively growing cells. When spores germinate, the viruses can reactivate and resume infection.

Three complementary approaches are used to understand this process. First, CRISPR-based viral genome editing and single-cell measurements are applied to test how specific viral genes and DNA sequences influence whether viruses remain inactive or proceed with replication during sporulation. Second, changes in both host and viral gene expression are examined using single-cell transcriptomics and image-based analysis to understand how entrapment affects cellular development. Third, microfluidic platforms are used to directly observe infected spores over time, providing insight into their survival, reactivation, and overall fitness.

By combining these approaches with computational modeling, this work provides new insight into how viruses persist in microbial communities under fluctuating conditions. These findings have broad implications for microbiome dynamics and may inform applications such as phage therapy targeting dormant or hard-to-eradicate bacterial populations.

---

the urinary tract bacterial isolates of Peptinophilus obesi and Anaerococcuss p. encode a novel 17B-HSDH

Briawna Binion, University of Illinois Urbana-Champaign

Microbial steroid metabolism represents an underappreciated extension of the vertebrate endocrine system, with growing evidence that host-associated microbes contribute to the diversity and bioavailability of sex steroids within human tissues. Emerging studies have linked microbial androgen metabolism to urinary microbiome composition and to resistance to androgen deprivation therapy (ADT) in prostate cancer. While microbial pathways capable of converting steroid precursors such as cortisol to androgens, via the steroid-17,20-desmolase pathway, as well as DesG-mediated interconversion of androstenedione to testosterone have been reported, the diversity of enzymes mediating downstream androgen interconversion remains incompletely defined. Here, we investigate the androgen-forming capabilities of anaerobic bacteria from the male genitourinary microbiome, focusing on NADPH-dependent 17β-hydroxysteroid dehydrogenases (17β-HSDHs) that catalyze interconversion of androstenedione and testosterone. We isolated androgen-forming bacterial strains from human male urine and identified a previously uncharacterized 17β-HSDH encoded by *Peptoniphilus obesi*, demonstrated that this enzyme catalyzes the NADPH-dependent reduction of androstenedione to testosterone and the reverse oxidation reaction. Sequence similarity searches further identified a homologous 17β-HSDH in *Anaerococcus*, which was synthesized and functionally validated, revealing conserved activity despite low sequence identity to the previously characterized urinary tract enzyme DesG. The enzymes were found to have broad substrate specificity for C19 and C18 17keto- and 17β-hydroxysteroids. Together, these findings expand the known diversity of microbial 17β-HSDHs and identify previously unrecognized androgen-forming activities within the genitourinary microbiome.

Importance: Microbial steroid-transforming pathways may provide a mechanism by which commensal anaerobes contribute to androgen availability in the genitourinary tract. By identifying novel 17β-hydroxysteroid dehydrogenases from *Peptoniphilus* and *Anaerococcus*, genera repeatedly associated with prostate cancer, this study provides mechanistic insight into how microbial steroid metabolism may influence hormone-driven disease.

Metagenomic analysis of the impact of wastewater discharge on the nitrogen cycle in the Chicago area water ways

Tris Bakke, Rachel Poretsky

Department of Biological Sciences, University of Illinois at Chicago

The nitrogen cycle is a critical nutrient process that involves many nitrogen transformations which converts nitrogen into chemically available and inaccessible forms. Many of these processes are completed by only bacteria and archaea in both terrestrial soil and aquatic environments. Treated wastewater contains high concentrations of nitrogenous compounds which are released into bodies of water and have the potential to have a significant effect on the composition of the functional microbial community. While previous work has focused on the nitrogen cycle in terms of wastewater treatment and remediation efforts, there is limited research on the impact of discharged effluent wastewater on the microbial functional gene profile of the nitrogen cycle.

The Terrance O'Brien (OB) Wastewater Reclamation Plant (WWRP) serves the northern suburbs of the Chicago metropolitan area which releases into the Northshore channel, which eventually leads to the Chicago River. While WWRPs attempt to remove large amounts of nitrogenous compounds from the water supply there is still a noticeable amount released in the effluent wastewater. To discover the impact of these compounds being released, we sampled at four sites over the course of a year, bimonthly. The sample sites were raw influent and treated effluent of the WWRP and upstream and downstream of the effluent discharge in the Northshore channel. We extracted DNA from both water and sediment samples for metagenomic sequencing. This analysis provided insight into the environmental impact of wastewater discharge on the freshwater nitrogen cycle. Our preliminary findings have shown a consistent decrease in nitrogen cycle genes downstream of the effluent in the water samples. Furthermore, the population of nitrogen cycle genes are much higher in the sediment comparatively to the water samples. These findings suggest the effluent wastewater restructures the microbial community composition.

Effect of passion fruit seed flour (Passiflora edulis) on intestinal health and metabolic parameters in normal and obesity adult mice

Andressa de Assis Lima[a], Danúbia Joanes Rosa Guerra Cabral[a], Pietra Vidal Cardoso do Prado[a], Renata Celi Lopes Toledo[a], Frederico Augusto Ribeiro de Barros[b], Thaisa Moro CantuJungles[c], Bárbara Pereira da Silva[a], Hércia Stampini Duarte Martino[a a] Nutrition and Health Department. Federal University of Viçosa [b]Department of Food Technology. Federal University of Viçosa [c] Department of Food Science. Purdue University, West Lafayette, USA

Obesity is associated with the development of metabolic disorders, and the intake of dietary fiber and phenolic compounds may modulate these metabolic changes, or prevent the development of obesity. Agro-industrial residues have emerged as important sources of bioactive compounds and may represent sustainable alternatives. Passion fruit processing generates by-products such as passion fruit seed flour (PFSF), that represents approximately 6–12% of the total fruit weight and presents a high content of dietary fiber (74.23g/100g) and total phenolic compounds (113.54GAE/g), especially piceatannol (45.7 µg/g), making it a potential ingredient for the development of value-added co-products. The aim of this study was to evaluate the effect of PFSF on intestinal health and metabolic parameters in vivo. C57BL/6 mice fed (8 weeks) a standard AIN-93 diet (AIN), and a high-fat diet (HF) to induce obesity. In intervention phase (8 weeks), the animals were divided into four groups (n=12/group): AIN, AIN + PFSM, HF and HF + PFSF. The AIN + PFSM increased total short chain fatty acid (SCFA), such acetic acid production, decreased glucose levels. HF + PFSF did not change total short chain fatty acid, but decreased butyric acid production, length of the small and large intestine and full cecum weight. PFSF added in AIN and HF diet did not change intestinal permeability, but decreased final body weight loss and adiposity. Further HF+PFSF decreased body mass index. It is concluded that passion fruit seed flour improved acetic acid production, decreasing glucose levels, body weight and adiposity in mice fed a standard diet. In obesity mice fed a high fat diet the passion fruit seed flour demonstrated better effects in modulating body weight and adiposity.

Molecular detection of Powassan Virus in Northern Illinois tick populations

Aurora Marguccio, Lance Jones, Chang-Hyun Kim, Chris Stone

Department of Integrative Biology, University of Illinois at Urbana-Champaign

Powassan virus (POWV, *Flaviviridae*: *Flavivirus*) is the only known North American tick-borne flavivirus that can cause severe encephalitis in humans. POWV consists of two distinct genetic lineages, specifically lineage 1 (prototype POWV virus) and lineage 2 (deer tick virus). Present in small- and medium-sized mammals, POWV is transmitted by three prominent tick species: *Ixodes scapularis*, *Ixodes cookei*, and *Ixodes marxi*. The virus is spread to humans by the bite of an infected tick. Once infected, about half of human host survivors may show long-term health problems, including physical impairment and neurological sequelae. In the US, historic data shows an increasing number of reported cases from 2004 to 2014 and an apparent rise in the past few years. Because POWV virus disease has historically been underdiagnosed, this spike may partly reflect recently improved arboviral surveillance and awareness rather than increased emergence alone. To better define its distribution, we tracked and documented the presence of POWV within natural areas in Illinois, a state with no prior detections before 2025, by surveying tick populations likely to sustain enzootic transmission. Individual *Ixodes scapularis*

samples were screened for POWV using real-time RT-PCR, and positive ticks were confirmed using conventional PCR followed by Sanger sequencing. A number of positive specimens were detected in Winnebago and Jo Daviess Counties, with infection prevalence higher in males than in females. Phylogenetic analysis of POWV-infected ticks showed that the viral sequences identified in Illinois form a distinct monophyletic clade within lineage 1. These findings support ongoing diversification of POWV following its introduction into the U.S. and underscore the importance of continued surveillance and proactive risk assessment to mitigate the potential for human outbreaks.

Engineering antibody fragment pharmacokinetics for improved in vivo microbial delivery

Yishuo Jiang, Vince W. Kelly, Shannon J. Sirk

Department of Bioengineering, University of Illinois Urbana-Champaign

Single-domain antibodies (sdAbs) are antibody fragments that retain the antigen specificity and affinity of full-length antibodies but can be more easily produced in prokaryotic cells. One limitation of sdAbs is their short serum half-life due to their small size (15 kDa), which is below the renal filtration cutoff of ~50 kDa. We aim to address this limitation using two orthogonal techniques: sustainably delivering sdAbs from gut commensal microbes and engineering sdAbs with reduced kidney clearance, thereby improving serum half-life. Another limitation of sdAbs is that they lack an Fc domain and hence cannot recruit immune responses against the disease target. To overcome this, we propose to introduce additional modifications to sdAbs. These modifications will facilitate engagement with the FcγRs of immune cells, triggering antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) against diseased target cells. By improving the pharmacokinetics and pharmacodynamics of sdAbs, we hope to enhance their efficacy, particularly when delivered in vivo by engineered living therapeutics.

Effect if sIgAs supplementation on In-vitro fermentation of HMOs by of breast fed and infant formula fed infant gut microbiomes

Marcelo Guerrero, Purdue University

Optimizing for the factors that influence development during infancy is thought to be important to set the tone for good health later in life. Diet, for example, influences gut physiology to raise ecological and immunological barriers against colonization of bacterial pathogens. Additionally, it extends the baby's ability to metabolize compounds such as human milk oligosaccharides (HMOs), for energetic, nutritional and physiological benefits. In this research, we standardized microbiological protocols to study how the gut ecologies of babies differ in their

ability to degrade HMOs in anoxic *in vitro* fermentations and to determine if fermentation kinetics are modulated by presence of human sIgA. We evaluated our findings by measuring pH, gas production (PSI overpressure), short-chain fatty acids (SCFA), organic acids and 16S amplicon sequencing throughout a period of 24 hours. Preliminary results show that the microbiomes of infants tend to cluster into high and low fermentation groups in terms of their capability to degrade HMOs. Additionally, IgA may slightly decrease the metabolization of HMOs into pro-inflammatory compounds and change gene expression profiles. We expect that this study will give insight into the ecological mechanisms that influence the degradation of HMOs in the infant gut and identify the metabolic outputs that may be generated in the process. Consequently, this information will inform medical professionals, nutritionists and industry leaders to consider these factors in their recommendations and formulations of formula products to optimize for adequate infant nutrition.

Longitudinal analysis of spectinomycin resistance dynamics in the piglet gut microbiome

Saralexis Torres[1], Sanjana Jose[2], Kaitlyn M. Sommer[3], Ryan Dilger[3], Rachel Whitaker[2,4]

[1] Informatics Programs, University of Illinois at Urbana-Champaign, Urbana, USA.
[2] Carl R. Woese Institute of Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[3] Department of Animal Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801, USA
[4] Department of Microbiology, University of Illinois, Urbana-Champaign, Urbana, IL 61801, USA

Prevention of antibiotic resistance (AR) in livestock production is critical for both animal and human health. The increasing emergence of multi-drug resistance phenotypes has become a significant concern within agriculture, leading to increased mortality rates and treatment costs. Bacteria within the order Enterobacterales are often implicated in the spread of AR, making them a major focus of research. While the molecular mechanisms of resistance and gene transfer are of increasing interest, their dynamics within complex microbial communities remain unclear. In this preliminary study, we monitored a cohort of 12 newborn piglets from 2 to 37 days old. Half of the piglets received SpectoGard (spectinomycin) treatment orally once on day 9, and we assessed changes in their microbial communities over time. Our objectives are to identify AR genes that increase in frequency due to treatment and to elucidate their mechanisms spread within the microbial community, while tracking their prevalence and persistence over time. We plan to track these genomic changes by collecting representative isolates at different time points. A planned subset of isolates will undergo growth-curve phenotyping (OD-based kinetics) in the presence and absence of

spectinomycin and will be categorized into resistance phenotypes using growth metrics such as area under the curve (AUC) and lag time. Longitudinal patterns will be assessed by comparing within-isolate growth under selection across time points and between treated and control groups. In parallel, MIC testing of high-resistance isolates and targeted genetic characterization are underway to identify trackable resistance determinants. This pilot study aims to establish a scalable workflow linking longitudinal phenotypes to resistance genetics in neonatal swine and to support future sequencing-based resolution of resistance mechanisms and persistence.

Denoising genomes with a phylogenetic empirical Bayes Model

Stephanie N. Majernik[1], Patrick H. Bradley[1,2,3]

[1]Dept. of Microbiology, The Ohio State University, Columbus, OH 43210 USA
[2]Infectious Diseases Institute, The Ohio State University, Columbus, OH 43210 USA
[3]Center of Microbiome Science, The Ohio State University, Columbus, OH 43210 USA

Large-scale metagenomic assembly efforts have dramatically increased the number of documented species with available genomes in databases. However, Metagenome Assembled Genomes (MAGs) are sourced from mixed communities, and can therefore introduce contamination, defined as genes from the wrong species or strain. The most common methods for addressing this issue simply remove genomes with significant estimated contamination (e.g., above 5%), but this still leaves some contamination in the remaining high-quality MAGs. The extent of this contamination may also be underestimated by common tools like CheckM. Worse, gene-level analyses may enrich for contaminants: in our prior analysis of a metagenomic case-control study of cirrhosis, we found that 38% of genes with significant differential detection were (likely) contaminants. We previously developed a tool, PanSweep, that predicted contamination by (1) using the predicted taxonomic range of each significant gene and (2) correlating gene and species counts across samples. While both methods were effective, the second requires access to metagenomes that include the genes and species of interest, and also requires reads to be mapped to both, which is resource-intensive. In contrast, predicting taxonomic range requires no additional data other than a tree. However, our previous tool used coarse-grained, approximate taxonomic information, and did not use other potentially-informative data, such as the number of times a gene was detected, or the predicted completeness/contamination of the genomes where it was found. We wanted to determine whether a more sophisticated test including detailed phylogenetic information could accurately predict MAG contaminants at scale. Here, we present preliminary results from an empirical Bayes model that uses phylogenetic information to identify likely contaminants. This model can be applied to novel MAGs or existing collections to

“denoise” likely contaminants and thus improve the reliability of downstream analyses.

Design of a soluble fiber mixture to promote complementary beneficial bacteria and consistent responses across individuals

Narakorn Tanasupawimon, Thaisa Cantu-Jungles, Bruce Hamaker

Department of Food Science, Purdue University

Dietary fibers play a key role in modulating the gut microbiota; however, inter-individual variability often limits their consistent efficacy across individuals. This study aims to design a soluble fiber mixture to promote the growth of complementary beneficial gut microbial groups while providing a consistent response across donors. The investigation consists of three *in vitro* fecal fermentation trials. An array of soluble fibers was fermented using a pooled fecal sample from three healthy donors. Short-chain fatty acids (SCFAs) were quantified, and microbial DNA was extracted for sequencing and bioinformatic analysis to evaluate microbial composition and metabolic output. Based on these data, fibers were selected according to criteria of outcome improvements including the promotion of beneficial complementary gut communities, as well as tolerability as inferred from fermentation kinetics and consistency. Selected fibers were combined into five possible mixtures. Next, the mixtures, along with the individual corresponding fibers, were subjected to the same fermentation protocol. We found that soluble fiber blends could promote a broader range of healthassociated taxa compared to isolated fibers, such as *Lachnospiraceae*, *Ruminococcaceae*, and other Clostridium cluster XIVa and IV species. Based on these outcomes, the optimal mixture to increase SCFA production and enhance complementary beneficial gut microbes will be selected. Finally, the optimal mixture and its individual fiber components will be fermented using fecal samples from ten healthy individual donors separately to evaluate inter-individual response consistency. We hypothesize that tolerable soluble fiber mixtures can be designed to shape the gut microbial community in a consistent and complementary manner, supporting their application for human health and incorporation into food products.

Effects of random, dilution-based subsampling of the gut microbiome on host outcomes in the American cockroach

Sophia Stokes, Kayla Cross, Victoria Barnette, Torey Hilbert, & Zakee Sabree

Department of Microbiology, The Ohio State University

Complex gut microbiomes have been found to be major drivers of animal development, physiology, and tissue differentiation, but it remains unclear whether normal host outcomes require the full microbial community or only a reduced subset of host-supportive taxa. The American cockroach, Periplaneta americana, is a useful model for studying these interactions due to its complex microbiome and ability to be reared germ-free without antibiotics. Cockroaches lacking their normal microbiome have demonstrated delayed development, smaller body size, and abnormal gut tissue development. While the effects of removing the gut microbiome on the host have been studied extensively, the effects of randomly subsampling the microbiome through dilution and excluding rare taxa have not yet been examined.

In this study, we investigated the effects of random, dilution-based subsampling of the microbiome on the host by inoculating germ-free cockroach nymphs with complete or 10-2 diluted microbial communities. We assessed host outcomes by examining body size, gut morphology, microbial community composition, and host transcriptional responses to the subsampled community. Our results indicate modest changes to host physiology in response to the diluted microbiome, with treatment reducing host body mass but not significantly affecting gut morphology. Host gene expression also differed between treatments, with complete microbiomes associated with increased host expression of functions related to growth and stability. We further compared normal- versus abnormal-presenting hosts after diluted-community inoculation to gain insight into host-supportive taxa. Lastly, we characterized changes in the microbial community during gut passage from initial feeding to feces, highlighting community restructuring and habitat filtering within the host digestive tract. Overall, these results provide insight into how microbiome subsampling affects host physiology and establish a foundation for future experiments aimed at identifying host-supportive microbial communities through stronger dilutions.

Investigation of bacterial biofilm formation mechanisms

Gamage Don, H.[1], Periyannan G.[2], Nathan, B.[1], Jayasooriya, S.[3], Satkunam, P., Sivakumar, P.[3]

[1]Department of Chemistry and Biochemistry, Eastern Illinois University
[2]Department of Biological Sciences Eastern Illinois University
[3]School of Physics and Applied Physics, Southern Illinois University Carbondale

The rapid emergence of antibiotic-resistant bacteria, largely driven by biofilm-mediated survival, necessitates a deeper understanding of the molecular mechanisms underlying biofilm formation. Biofilms constitute structured microbial communities embedded within an extracellular polymeric substance (EPS) matrix, which enhances resistance to antimicrobial agents and environmental stressors. In

this study, *Caulobacter crescentus*, a non-pathogenic and metabolically versatile alphaproteobacterium, was utilized as a model organism to investigate biofilm formation under chemical stress imposed by toxic aromatic hydrocarbons.

The primary objective was to evaluate the biofilm-forming capacity of *C. crescentus* in the presence of benzaldehyde, protocatechuate, and phthalate as sole carbon sources, and to characterize the biochemical and structural properties of the resulting biofilms. Bacterial growth was quantitatively assessed using optical density measurements and colony-forming unit (CFU) enumeration, while microscopic analyses provided insights into biofilm architecture. Highperformance liquid chromatography (HPLC) was employed to determine substrate utilization dynamics, revealing differential uptake patterns among the tested carbon sources.

The EPS matrix was comprehensively characterized using Fourier-transform infrared (FTIR) and Raman Spectroscopy along with quantitative analyses of uronic acids, proteins, and carbohydrates, thereby demonstrating carbon-source–dependent variations in biofilm composition. In addition, cellular envelope adaptations were investigated by isolating and analyzing lipopolysaccharides (LPS) and outer membrane proteins in planktonic cells. The impact of aromatic hydrocarbon–induced stress on regulatory pathways was further examined by assessing extra cytoplasmic function (ECF) sigma factor expression using RT-PCR.

Collectively, these findings elucidate key molecular and physiological adaptations that facilitate biofilm formation under aromatic hydrocarbon stress. This study advances our understanding of microbial metabolic flexibility, biofilm-associated antibiotic tolerance, and stress-responsive regulatory mechanisms, establishing *C. crescentus* as a robust model for investigating biofilmdriven resilience in toxic and nutrient-limited environments.

Examining microbial fitness drivers in the legume-rhizobia mutualism

Murray, H. C., Heath, K. D.

Legumes form a facultative mutualism with nitrogen-fixing bacteria (rhizobia) and provide essential ecosystem and agricultural services. Rhizobia fix atmospheric nitrogen in excess for the plant, which is then deposited into the soil, thereby increasing an often-limited resource. Our aim is to conserve the symbiosis, but this is hindered by a limited understanding of how rhizobia interact with host plants, conspecific and heterospecific microbes, and abiotic conditions on an ecological scale. When rhizobia are in symbiosis within specialized plant organs called nodules, rhizobia receive carbon from the host plant, which facilitates the expression of metabolically expensive nitrogen fixation genes, providing biologically accessible nitrogen for the plant. The model legume *Medicago truncatula* allocates more carbon resources to *S. meliloti* partners that produce greater amounts of nitrogen, resulting in increased population sizes of these beneficial partner quality

strains[1]. Curiously, a wide diversity in partner quality of rhizobial strains persists in nature despite this selective pressure. Conflicting drivers of fitness outside of the nodule environment could explain the maintenance of partner quality variation.

Here, I try to disentangle the benefit that Sinorhizobium provides by investigating their interactions with legumes beyond nodulation. Using two Medicago genotypes (a nodulation defective mutant and wild type A17) inoculated with different combinations of Sinorhizobium strains we asked if we could isolate a benefit of Sinorhizobium inoculation without the nodules– is there a rhizosphere benefit? We found that without the symbiotic relationship, there is marginal detriment which is conferred to the plant. Strains which exhibited a gradient of partner quality in the nodules, performed identically in the rhizosphere.

Elucidating the hierarchy of necromass degradation in a marine sediment-derived microbiome

Paul Lee[1], Hiroyuki Imachi[2], Masaru K. Nobu[2], Ran Mei[1,2*]

1. Department of Civil and Environmental Engineering, University of Illinois Urbana-Champaign, Illinois, USA
2. Institute for Extra-cutting-edge Science and Technology Avant-garde Research (X-star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Japan

Deep-sea sediments represents a major reservoir of organic carbon and nitrogen, and sediment microbiomes play central roles in cycling organic matter derived from decaying biomass, i.e., necromass. Necromass is compositionally heterogeneous, consisting of proteins, lipids, and polysaccharides, and its degradation requires coordinated activity across diverse microbial populations. At the same time, sediment microbial communities are themselves highly diverse, comprising phylogenetically and metabolically distinct lineages. Despite this dual complexity, current models of necromass degradation largely treat the process as bulk hydrolysis mediated by freely secreted extracellular enzymes, without resolving how different organisms contribute to distinct steps of substrate breakdown and assimilation.

Here, we leveraged a long-term (20 years) bioreactor seeded with deep-sea sediment and spiked the microbial community with a gradient of proteinaceous substrate complexity in batch cultures. We combined genome-resolved metagenomics and metatranscriptomics, and integrated protease localization with transporter expression to define a functional space describing macromolecule degradation mode (extracellular vs. membrane-associated) and substrate uptake preference (large peptides vs. amino acids and oligopeptides).

We found that proteolytic activity is not dominated solely by extracellular enzymes. Instead, a substantial fraction of expressed proteases are predicted to be surface-associated, indicating that protein degradation frequently occurs in close proximity to the cell rather than only through freely diffusible enzymes. These results suggest that necromass degradation in deep-sea sediments is functionally partitioned across microbial populations, with membrane-associated proteolysis representing an underappreciated strategy for accessing organic matter under energy-limited conditions. This framework links the compositional complexity of necromass to the functional organization of microbial communities and provides new insight into carbon turnover in the deep biosphere.

Using ex vivo oral biofilms from saliva to identify bacteria targeted by polyphenols

Tamamidis, Michaela; Lopez, Anthony; Li, Wei; Wu, Christine; and Adami, Guy

Oral Medicine and Diagnostic Sciences Department, University of Illinois Chicago, Dentistry

Networks of different bacteria taxa contribute to oral health. Antibacterials ideally would selectively target the subset of bacteria contributing to disease. Plant-derived natural compounds have been shown to inhibit planktonic bacteria or monospecies oral biofilms. However, very few in vivo studies are available to demonstrate the effects of these antimicrobials against native biofilms found in the human oral cavity. The objective was to create a simple system to test putative antibacterials for their ability to target specific bacterial taxa in complex biofilms. Oral biofilms from donors were collected from saliva then rapidly prepared for polyphenol testing. They were then incubated with test polyphenols for 1 or 4 hours. 16s rRNA was measured using NGS cDNA sequencing to determine targets of bacterial inhibition. Five polyphenols, tested at physiological levels, showed inhibition of select oral bacteria that was reproducible across three timepoints. Some targets were individual-specific. Testing with oral biofilms from an additional 14 individuals revealed many taxa that were targeted consistently across the population. Of those tested, three polyphenols, kaempferol, myricetin and quercetin used at physiological levels inhibited oral bacterial species associated with both periodontal disease and cardiovascular disease while sparing many commensal bacteria taxa. Conclusion: Plant-derived polyphenols are a potential source of selective antimicrobial compounds against oral and systemic pathogens.

Ecological drivers of immune structure and viral escape in microbial populations with CRISPR

Yang, J.[1], Liaghat, A.[2], Cowell, T.[1], Pascual, M.[3], & Whitaker, R.[1]

[1] Department of Microbiology, University of Illinois Urbana-Champaign
[2] Department of Ecology and Evolutionary Biology, University of Michigan
[3] Departments of Biology and Environmental Studies, New York University

The CRISPR–Cas system encoded in archaeal and bacterial genomes consists of arrays of viral-derived sequences (spacers) acquired from past infections, and thus reflects virus-host coevolutionary history. Across various natural systems, array length and associated immune structure can vary significantly. In parallel, CRISPR-mediated coevolution *in vitro* is prone to rapid viral extinction, resulting in short host array lengths. The eco-evolutionary conditions underlying contrasting patterns of CRISPR array length and emergent immune structure remain unclear.

We reconstructed full-length CRISPR arrays using long-read sequencing from *Sulfolobus islandicus* populations sampled over 12 years from geothermal hot springs of Yellowstone National Park. Arrays were consistently long (47–53 spacers on average) and comprised only a few persistent trailer-end lineages (2–3). A rooted triplet-based CRISPR phylogeny revealed stepwise spacer acquisition at the leader end while preserving trailer-end structure.

Motivated by the low host-density conditions of these hot springs, we used a stochastic model to ask how host carrying capacity (K) interacts with spacer acquisition probability (q) jointly shape long-term virus–host coevolution, with K setting ecological constraint and q governing immune diversification. Numerical simulations revealed two regimes across q-K phase space. In a *high-control* regime characterized by many trailer-end lineages defined by the first spacers acquired, coevolution yields short times to viral extinction along with short array lengths. By contrast, in a *high-escape* regime with only a few lineages, coevolution yields prolonged times to viral extinction and long array lengths.

We analytically derive the expected number of lineages at the onset of coevolution as a nonlinear function of q and K, increasing with either parameter. Using this expression, we formulate the reproductive ratio of an initial viral escape mutant. This R_0 captures escape selection strength and increases with both times to viral extinction and array lengths, serving as a predictive measure of coevolutionary persistence.

Together our results suggest that the *S. islandicus* system resides in the high-escape regime consistent with low host-density conditions. Under these conditions, both co-evolutionary partners exhibit strong, recurrent selective sweeps, which allow for prolonged coevolution and array lengths that expand in a stepwise fashion. More broadly, our results provide a basis for identifying eco-evolutionary conditions at play through patterns of immune diversity and structure.

Core resistomes are shared across diverse One Health sources

Nhung Do[1,2,3], Yuqing Mao[1,2,3], Hien T.T Ngo[2], Andrew W. Taylor-Robinson[2,4], Nohra E. Mateus-Pinilla[5], Thanh H.(Helen) Nguyen[1,2,3]

[1]Department of Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign
[2]VinUni-Illinois Smart Health Center – VinUniversity
[3]Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign
[4]College of Health Sciences – VinUniversity
[5]Illinois Natural History Survey (INHS) - Prairie Research Institute

Introduction: The environmental dissemination of antimicrobial resistance (AMR) is a global health priority; however, many clinically relevant antibiotic resistance genes (ARGs) remain undetected in complex environmental samples due to their low abundance. This study utilizes a "One Health" framework to determine whether clinically-relevant ARGs previously found mainly for humans circulate across human, animal, and water compartments.

Aim: We investigate the distribution of resistant markers, especially targeting OXA beta-lactamases and co-occurring genes across clinical human settings, wastewater treatment plants (WWTP), and migratory Canada Geese (*Branta canadensis*) in Illinois, US.

Methods: We employ an improved CRISPR-NGS enrichment approach to overcome the limitations of standard metagenomics in detecting low abundant ARGs. Then, qPCR assay is used for resistant markers quantification. These two complementary methods provide quantitative information of the co-occurence for clinically relevant ARGs in these One Health matrices.

Results: The CRISPR-NGS approach significantly broadens the detectable resistome revealing a diverse array of Acinetobacter-associated OXA variants and associated MDR genes. In addition, we identified a conserved core resistome resistant to tetracyclines, cephalosporins, fluoroquinolones, and aminoglycosides that were consistently present across clinical, wastewater, and avian samples.

Conclusion: The overlap in resistance to four major antibiotic classes indicates that anthropogenic resistance markers have become established in the environment and underscores the role of migratory species as recipients and carriers of ARGs.

Public health relevance: By using an innovative methodology consistently across a diverse set of One Health samples, we found evidence of shared OXA beta-lactamase families in geese environmental fecal samples, demonstrating that these migratory species pick up dangerous ARGs, and may play an important role in sharing them across One Health environments.

Synergetic SCFA increase in gut microbiota in healthy and diseased individuals by fiber mixtures

Gabriel Santiago Galeano-Garcia
Purdue University

Dietary fibers promote short-chain fatty acid (SCFA) production, but the combined effects of fiber mixtures remain understudied. This study investigated synergistic SCFA production from a four-component fiber mixture (pectin, β-glucan, fructooligosaccharides, arabinoxylan) using *in vitro* fermentation data from individuals across four health conditions: healthy controls, Parkinson's disease, Crohn's disease, and ulcerative colitis. The fiber mixture synergistically boosted total SCFA production by 32.8% ± 20.1% beyond expected levels across all conditions. Acetate and total SCFAs increased consistently, with condition-specific increases for propionate in Parkinson's disease and butyrate in ulcerative colitis and healthy controls. Individuals exhibiting synergy were enriched in taxa from Lachnospiraceae and Ruminococcaceae families, key butyrate producers that likely facilitate cooperative interactions. Synergy was not correlated with microbial diversity or absolute SCFA amounts. These findings suggest strategically designed fiber mixtures may be more effective prebiotics than individual fibers by supporting cooperative microbial interactions across health conditions.

Protein removal modifies arabinoxylan structure and fermentation kinetics

Raghunath, R., Lindemann, S. R.

Department of Food Science, Purdue University

Arabinoxylan (AX), a polymeric hemicellulose with arabinofuranose (Ara*f*) substituted on a xylopyranose (Xyl*p*) backbone, is the predominant dietary fiber across most cereal brans. Extraction and isolation of AX from the bran matrix involves enzymatic starch and protein removal, followed by alkaline and peroxide-mediated ester hydrolysis to delignify and liberate cross-linked AX from the lignocellulosic network. Residual protein often persists as protein-AX complexes due to co-localization and co-extraction with polysaccharide structures. Protein removal with proteases and organic solvents can hydrolyze peptide bonds, however, their specific interactions with fine fiber structure, and consequently, downstream gut microbiome interactions will need to be studied. We tested the hypothesis that protein removal may alter AX structure and gut fermentation patterns, namely short-chain fatty acid (SCFA) metabolite production.

We treated three AXs each derived from wheat, corn and sorghum bran first with proteinase-K (PK) enzyme (1.2 mg/mL, suspended in 1% SDS) and a sequential phenol-chloroform-isoamyl alcohol extraction (PCI, 25:24:1, v/v). This was followed by a second chloroform-isoamyl alcohol extraction (CI, 24:1, v/v). The treated AXs were

compared to untreated controls. We measured protein content by dot-blotting and Dumas nitrogen analysis, neutral monosaccharides and glycosidic linkages by alditol acetate derivatization in GC-MS, molecular weight (MW) using size-exclusion chromatography, and crystallinity using X-ray diffraction (XRD). Treated AXs did not significantly differ in MW, neutral monosaccharide and glycosidic linkages, however crystallinity analysis indicated intermolecular alterations occurred in a cereal source-dependent manner. Fermentation for 48 hours with fecal microbiota from three donors revealed higher pH, slower gas production, lower SCFA production, and divergent taxonomic composition, again in a cereal source-dependent manner. Overall, these results may offer strategies to tailor dietary fibers and achieve specific structural configurations for desired physiological outcomes.

Establishing experimental foundations for studying biofilm invasion mechanisms in drinking water systems

Erick J. Negrón Álvarez, Caitlin R. Proctor

School of Sustainability Engineering and Environmental Engineering, Department of Agricultural and Biological Engineering, Purdue University

Microbial life in drinking water systems is predominantly associated with biofilms, where microorganisms can persist, interact, and in some cases support the survival of opportunistic pathogens such as *Legionella pneumophila* and *Pseudomonas aeruginosa*. Traditional control strategies rely on disinfection, yet these approaches are often insufficient in plumbing systems, as biofilms continue to grow. A novel approach to managing drinking water microbial exposure is to engineer stable microbial communities.

It is first critical to establish experimental and analytical methods to study biofilm formation and microbial dynamics in engineered water systems. Invasion is a key parameter of interest, as an ideal stable community will prevent invasion by potential pathogens. Model organisms, including *Pseudomonas fluorescens*, are being used to develop reproducible invasion assays under controlled laboratory conditions. Growth dynamics can be monitored through optical density measurements and colony morphology assessments to ensure culture consistency and purity.

To investigate biofilm structure and invasion, confocal laser scanning microscopy (CLSM) is being implemented to visualize spatial organization and assess variability in early biofilm development. In parallel, flow cytometry (FCM) is being explored as a tool for rapid quantification of microbial populations, and molecular methods including PCR are being developed to support future community-level analyses. *Pseudomonas fluorescens* will be used in these assays because it can be identified without staining, allowing it to be distinguished from indigenous bacteria, for rapid quantification of invasion.

Preliminary results demonstrate consistent growth behavior of model organisms and successful establishment of foundational workflows for microscopy and microbial quantification. These efforts provide a reproducible platform for future studies aimed at understanding how environmental conditions and initial colonization influence biofilm assembly and stability.

Ultimately, this work contributes to ongoing efforts to move beyond biofilm removal strategies toward approaches that leverage microbial interactions to promote stable and resilient communities in drinking water systems.

Degradation of Cyanobacterial extracellular polysaccharides in microbial communities

Toko Hisano[1] and Stephen R. Lindemann[1,2,3,4]

1 Department of Biological Sciences, Purdue University, West Lafayette, IN
2 Department of Food Science, Purdue University, West Lafayette, IN
3 Department of Nutrition Science, Purdue University, West Lafayette, IN
4 Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN

Cyanobacteria fix CO_2 and provide organic compounds to ecosystems. One major category of cyanobacterially produced organic compounds are extracellular polysaccharides (EPS). Cyanobacterial EPSs are typically heteropolysaccharides comprising a broad range of monosaccharides and functional groups; they also often possess branched chains. Even though degradation of cyanobacterial EPS is a part of the global carbon cycle, we still lack a comprehensive understanding of the degradation process of EPS in microbial communities. In this study, we focused on EPS extracted from *Cyanobacterium stanieri* HL-200, isolated from a hypersaline lake, Hot Lake in WA. This strain secretes a large amount of EPS, which is composed of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose. Here, we establish heterotrophic bacterial communities that degrade the EPS efficiently and investigate the cooperation of bacteria in the degradation of the structurally complex EPS. Environmental samples were collected from Hot Lake. Inocula from microbial mats and sediments with water were separately introduced into a medium containing EPS (1 g/L) as a sole carbon source and incubated at 30°C under aerobic conditions. The turbidity of the cultures increased within a week of cultivation, and the cultures were successfully subcultivated 6 times. Pinkish-colored flocks were observed in the enrichment cultures. The residual total sugars after 32 days of cultivation were less than 10%, as determined by the phenol-sulfuric acid assay. 16S rRNA gene amplicon sequencing was performed (V4-V5) and a total of 2,841,265–2,982,827 reads were obtained. The community structures of the two enrichment cultures were similar and were dominated by OTUs in the class *Phycisphaerae* sharing <87% identity with known species, with 10 to 15 OTUs

representing more than 1% of the total reads. We are currently isolating these bacteria and assessing their combined effects on EPS degradation.

Intestinal and blood microbiota-mediated remodeling of postpartum inflammation in dairy cows

D. G. Puerres Narvaez*[1], L. Das[1,2], N. Taechachokevivat[1], R. C. Neves[1], I. B. Slizovskiy[1,3,4]

[1]Department of Veterinary Clinical Sciences, Purdue University, [2] Department of Entomology, Purdue University,[3] Purdue Applied Microbiome Sciences Program, Purdue University, [4] Purdue Institute of Inflammation, Immunology and Infectious Disease, Purdue University.

The intestinal microbiota modulates host immunity and influences inflammation. In dairy cows, this interaction is critical during the postpartum transition, when prolonged inflammation affects longterm health. This study evaluates how an exacerbated postpartum acute-phase response is associated with changes in the fecal microbiome and potential bacterial signals in blood, while accounting for contamination and biological confounders.

We analyzed fecal and blood samples from 71 Holstein cows at 1 and 3 days in milk (DIM). Inflammation was assessed via haptoglobin and fibrinogen, considering parity, DIM, body condition score, and qPCR bacterial load. Negative and positive controls were included. The region V3–V4 of the 16S rRNA gene was sequenced; blood sequencing data underwent host-read removal using Bowtie2. Fecal and blood sequencing data were processed with DADA2, microbiome composition was analyzed in Phyloseq, and differential abundance was tested using DESeq2.

Contaminants in fecal samples were removed using *decontam* and SourceTracker, reducing the dataset from 24,011 to 21,727 ASVs. SourceTracker attributed most contamination to extraction blanks (99.61%). For blood samples, the dataset was reduced from 25,218 ASVs to 22,145 and 23,564 with Decontam and SourceTracker respectively. Following decontamination, beta diversity was significantly associated with inflammation, measured by fibrinogen and haptoglobin ($R^2 = 0.015$, $p = 0.001$) in fecal samples. Parity was the only significant factor in alpha diversity, with multiparous cows showing reduced richness and diversity. Future work will focus on community diversity analyses of blood samples following host-read removal, as well as assessing microbial translocation to further characterize microbiome dynamics and its association with inflammation.

Exploring the impact of the microbiome on bourbon fermentation efficacy and volatile production

Samuelson, Cooper[1]; Heist, Melanie[2]; Barzee, Tyler[3]; Moe, Luke[1]

[1]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY; [2]Ferm Solutions, Danville, KY; [3]Department of Biosystems and Agricultural Engineering, University of Kentucky, Lexington, KY

The unique flavor profiles that distinguish bourbon brands are predominantly formed from variations in mash bills, yeast strains, and maturation practices. Yeast strains generate the foundation of the flavor profile during fermentation. However, fermentation is not a sterile process, as other microorganisms colonize and persist in distilleries despite industrial cleaning procedures. We hypothesize that the collective microbiome within the fermentation ecosystem contributes to the overall success and chemical makeup of the resulting spirit. To determine the impact of bacteria, we inoculated fermentations with species commonly found in distilleries: *Limosilactobacillus fermentum* (formerly *Lactobacillus fermentum*), *Enterococcus faecalis*, *Pediococcus pentosaceus*, and *Acetobacter pasteurianus*. We assessed the impact of single species inoculations at two titers (10^6 and 10^8 CFU/mL) on fermentation efficacy and volatile production. A small-scale, high-throughput fermentation strategy was developed and optimized for consistency across batches, enabling parallel studies between yeast-only fermentation and those with bacterial inocula. A 70/20/10 corn, rye, malted barley grain bill was used to reflect the standard bourbon grain bill. A one-way ANOVA revealed that *L. fermentum, E. faecalis,* and *A. pasteurianus* at 10^8 CFU/mL had significantly lower concentrations of ethanol compared to the yeast-only control, along with further differences that varied both by bacterial species and titer. A two-way ANOVA, used to compare differences between the inoculated fermentations, showed that volatile production varies the most by species. For instance, isoamyl acetate, an ester with a banana aroma, was found to have the greatest concentration in samples inoculated with *P. pentosaceus*. Additionally, interaction effects were found between species and titer for some fermentation and volatile measurements. These results indicate that bacteria have a significant effect on fermentation and present opportunities for greater refinement of flavor within the alcoholic beverage industry.

Disentangling root-associated microbes in agricultural fields in Indiana and their influence on soil phosphorus levels.

Martinez, E. A. & Wilhelm, R. C.

Department of Agronomy, Purdue University

In ag fields with reduced synthetic phosphorus (P) inputs, microbial processes can supply a substantial portion of plant P demand. Interactions among roots, fungi, and bacteria are central to P provisioning, where fungi facilitate P transport, helper bacteria contribute to P transformation, and plants provide carbon sources. The relative influence of each partnership remains an active area of discovery, especially among different soil types and management scenarios (e.g., fertilization). This study

evaluated changes in soil P levels and microbial community structure across fifteen agricultural fields in Indiana under contrasting management systems (AgSpectrum vs. Others) and cropping systems (corn and soybean). Size-exclusion mesh bags were deployed to create gradients of biological access: 1000 µm (roots + fungi + bacteria), 45 µm (fungi + bacteria), and 1 µm (bacteria only). Soil P levels were quantified through mineral analyses (MIII and Bray P2), and microbial communities were characterized using 16S rRNA gene sequencing. These results provide a field-based baseline for understanding how plant-microbe interactions regulate P availability under real agricultural conditions. This work advances our understanding of the soil's natural capacity to supply P in an effort to develop more efficient uses of the natural capital of ag fields.

A designed prebiotic mixture reduces IBS symptoms More Than Probiotic Treatment in a Randomized Crossover Trial

Organski, A.C., Lazzaron, G., Campos, M.R., Keshavarzian, A., Hamaker, B., Rabito, E.I., Cantu-Jungles, T.

Department of Food Science, Purdue University

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by recurrent abdominal pain, bloating, and altered bowel habits. While the underlying mechanisms of IBS are not fully understood, evidence suggests a critical role for the gut microbiome in symptomology. Modulation of the gut microbiota through probiotics and prebiotics have shown potential benefit in improving IBS outcomes; however, the comparative effectiveness of probiotics versus prebiotics remains unclear.

OBJECTIVE: We aimed to assess a probiotic and prebiotic efficacy at improving IBS outcomes.

METHODS: Adults with constipation-predominant IBS (N=21) completed a double-blind randomized crossover trail receiving *Lactobacillus rhamnosus* and a designed prebiotic mixture containing resistant starch, rice bran, resistant maltodextrin, and branched inulin in separate 4-week periods, with a 4-week washout. IBS symptom severity was assessed using IBS Symptom Severity Scale. Gut microbiota was evaluated via 16S rRNA gene sequencing, and inflammation and intestinal barrier function were assessed using fecal calprotectin and serum lipopolysaccharide-binding protein (LBP) and zonulin.

RESULTS: Prebiotic intervention outperformed the probiotic in reducing overall symptom severity, with significant reductions in reported abdominal pain frequency, abdominal distension, dissatisfaction with bowel habits, and symptom-related interference with daily life, along with a trend toward reduced abdominal pain intensity (p=0.068). In contrast, probiotic treatment was associated with a

trend toward reduced abdominal distension (p=0.059). These symptom differences induced by prebiotic intervention were not accompanied by changes in calprotectin, LBP, or zonulin; however, probiotic intervention resulted in a significant reduction in zonulin. Neither the prebiotic nor the probiotic altered beta-diversity, although probiotic treatment led to a significant increase in alpha-diversity.

CONCLUSION: The designed prebiotic was more effective than probiotic intervention in reducing IBS symptoms; however, this was not accompanied by changes in gut microbiota or markers of intestinal barrier function. Collectively, these findings may help inform future therapeutic strategies for individuals with IBS.

Symbiont co-infection and exposure to warm temperatures modify the efficacy of Wolbachia-induced feminization in the dwarf spider, Mermessus fradeorum

Doremus MR, Williams E, and White JA

Department of Entomology, University of Illinois

Maternally-transmitted endosymbiotic bacteria infect most terrestrial arthropods, rely on maternal reproduction for their transmission, and can manipulate host reproduction to favor infected females. Various internal and external environmental factors may also shape the infection dynamics of heritable symbioses, with the presence of other co-infecting symbionts in the same host or abiotic stressors like temperature influencing the efficacy of symbiont-induced phenotypes and the stability of the symbiont community. Symbiont co-infections are particularly common in linyphiid dwarf spiders like *Mermessus fradeorum*, which is host to at least five different types of heritable symbionts. These include a cytoplasmic incompatibility-inducing strain of *Rickettsiella*, a strain of Ca. *Tisiphia*, and three strains of *Wolbachia*. In nature, ~25% of spiders are quintuply-infected with all five symbionts and are feminized so that genotypic males develop as phenotypic females. By comparing offspring sex ratios across nine different infection combinations ('symbiotypes'), we found that one *Wolbachia* strain is responsible for feminization. Feminization rates also increased by ~10% when hosts were co-infected with the feminizing *Wolbachia* and a second, non-feminizing Wolbachia strain. This synergistic effect on feminization may promote the spread of this co-infection, as the feminizing *Wolbachia* is rarely found in nature without the second *Wolbachia* strain. We next tested how temperature stress affects the *M. fradeorum* symbiont community by exposing developing spiders either to a benign temperature (20C) or a stressful temperature (28C). We did not observe any immediate effect of temperature on feminization in spiders exposed to warm temperatures; however, feminization rates were reduced in the offspring of heat-treated spiders. Feminization failure corresponded with a shift in the symbiont

community caused by the transmission failure of multiple symbionts. Feminization rates increased two generations removed from heat exposure, if spiders retained the feminizing *Wolbachia*. Temperature stress has multi-generational consequences for the *M. fradeorum* symbiont community and, along with interactions between co-infecting symbionts, likely shapes symbiont infection dynamics in nature. Research on how heritable symbionts respond to their environment should consider these multi-generational effects when studying how heritable symbionts will respond to climate change through this century.

Understanding the ocular surface microbiome and amniotic membrane proteome interaction

Capistrano, E.

Department of Comparative Biosciences, University of Illinois

Amniotic membrane is the avascular innermost layer of the placenta and has been used to treat several ophthalmic conditions affecting the ocular surface. It has been shown that AM contains several proteins that enhance corneal healing, but its interaction with the ocular surface microbiome, particularly in certain diseases, remains unclear. This study aims to associate the reported ocular metagenome with the bovine amniotic membrane proteome, using Gene Ontology (GO).

Methods: Bovine amniotic membranes (BAM) (n =10) were acquired from normal full-term births, processed, and stored at - 80 °C for two days. After thawing, the BAMs were prepared for high-resolution liquid chromatography-mass spectrometry, followed by bioinformatic analysis. The protein profiles and GO present in BAM were compared with the ocular surface metagenome described in the literature.

Results: In humans, among the bacterial diversity in the eye, the most dominant phyla described were *Actinobacteria*, *Proteobacteria*, *Firmicutes* with *Corynebacterium*, *Propionibacterium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Acinetobacter*, and *Agrobacterium.* In our study, a total of 2,015 proteins were identified in the BAM, of which GO annotations were available for 1,472. The GO annotations of importance correlated with the bacterial diversity identified in the human eye were fatty acid-binding protein (correlated with *Firmicutes*), antimicrobial humoral response (correlated with *Agrobacterium*), and inflammation mediated by cytokine (correlated with *Corynebacterium* and *Propionibacterium*).

Conclusion: BAM expresses proteins whose GO correlate with the dominant phyla in the ocular surface, suggesting that amniotic tissue may influence the ocular microbiome and could be leveraged strategically for ocular surface-associated diseases.

Ultra-marathon running reshapes gut microbial aromatic amino acid metabolism and disrupts barrier function

Casey KY. Lim[1], Mikaela C. Kasperek[2], James R. Bagley[3], Gregory J. Grosicki[4], Jacob M. Allen[1,2]

1. Department of Health and Kinesiology, University of Illinois at Urbana-Champaign, 906 S. Goodwin Ave, Urbana, IL 61801
2. Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, 906 S. Goodwin Ave, Urbana, IL 61801
3. Department of Kinesiology, San Francisco State University, 1600 Holloway Avenue, GYM 101, San Francisco, CA 941324
4. Department of Performance Science, WHOOP, Inc, 1 Kenmore Square, Boston, MA 02215

BACKGROUND: The gut microbiome plays a central role in the metabolism of aromatic amino acids (ArAA), producing bioactive metabolites through both oxidative and reductive pathways. Microbial metabolism is dynamic and responsive to physiological stress that disrupts the gut microbiome and epithelial integrity. Whether this is associated with shifts in ArAA metabolism toward oxidative or reductive pathways that relate to barrier function remains unknown.

PURPOSE: To characterize pre- to post- ultra-endurance race changes in gut microbial composition, ArAA-derived metabolites, and signs of barrier disruption in participants of the 2023 Western States Endurance Run (100 miles).

METHODS: Runners (n=37) provided pre- and post-race stool (≤72 h) and serum (<1 hr). Gut microbiome composition was analyzed using 16S rRNA sequencing, including measures of α- and β-diversity and relative abundance of *Streptococcus*. Circulating and fecal ArAA metabolites were quantified via targeted LC-MS and grouped as oxidative (aryl-acetates) or reductive (aryl-lactates), reflecting divergent microbial redox pathways. Intestinal epithelial injury and barrier disruption were assessed by plasma intestinal fatty acid–binding protein (I-FABP; enterocyte damage marker) and lipopolysaccharide-binding protein (LPS-BP; marker of systemic endotoxin exposure).

RESULTS: Microbial α- and β-diversity were unchanged after ultra-marathon; however, *Streptococcus* abundance increased ($p = 0.0083$). Circulating oxidative aryl-acetates increased ($p = 0.0057$) while reductive aryl-lactates decreased ($p = 0.0039$), supporting a shift toward oxidative microbial metabolism. Fecal aryl-acetates and aryl-lactates were not significantly altered ($p = 0.1926$, $p = 0.0721$), although the overall directional pattern was consistent. I-FABP and LPS-BP increased post-race ($p = 0.0245$, $p = 0.0001$). There was a positive correlation between I-FABP and race time was $\rho = 0.49$ ($p = 0.078$), indicating a relationship between extreme exercise performance and gut barrier integrity.

CONCLUSION: Acute ultra-marathon participation associates with shifts in gut microbial ArAA metabolism toward oxidative pathways without broad changes in overall microbial diversity, supporting functional changes in microbial amino acid metabolism under extreme physiological stress.

Precise control of outer membrane vesicles in a human gut commensal

Tarabey, M & Sirk, S.

Department of Bioengineering, University of Illinois Urbana-Champaign

Protein-based therapeutics, including monoclonal antibodies and therapeutic enzymes, represent a powerful and highly specific class of interventions for the treatment and prevention of numerous diseases. However, protein therapeutics face substantial limitations including high manufacturing costs, the need for repeated systemic administration, and poor accessibility to certain anatomical sites such as the gastrointestinal tract. Engineered living therapeutic (ELT) platforms offer a promising alternative approach to overcome the challenges of conventional protein-based therapeutics. ELTs consist of engineered microbial cells designed to prevent or treat disease from within the host, enabling sustained, *in situ* production and delivery of therapeutic molecules.

To achieve our goal of producing persistent ELTs, we chose to engineer a prominent human gut commensal microbe, *Bacteroides thetaiotaomicron (Bt)* to produce outer membrane vesicles (OMVs) as delivery vehicles for therapeutic proteins. OMVs are nanoscale (50-250nm diameter), spherical lipid bilayer vesicles produced and secreted by all Gram-negative bacteria. The vesicular nature of OMVs provides enhanced protection of cargo proteins from environmental proteases and increased resistance to extreme pH conditions– advantages that are particularly attractive for therapeutic delivery within the gut.

Building off previous work in our lab, we identified native bacterial secretory sequences in *Bt* which enable recombinant protein loading into OMVs. Utilizing proteinase K accessibility assays and split protein reporter systems, we found that different native secretion pathways in *Bt* will package proteins into different locations within an OMV (either on the surface or in the lumen). This study aims to leverage our understanding of protein loading within *Bt* to create a genetically encodable, biological lipid nanoparticle platform which allows user-defined control of therapeutic protein concentration and orientation within OMVs.

Polysaccharide utilization loci link dietary fiber degradation to metabolite profiles in human gut Bacteroides

Alhawsawi, M. AB., Abdel Hamid, A. M., Bianchi, D. M., Akresi, J., Robinson, A., Pereira2, G.V., Hess, R. Y., Mackie, R. I., & Cann, I.

Division of Nutritional Sciences, University of Illinois Urbana-Champaign

Microbiota produce various carbohydrate-active enzymes (CAZymes) to partially or completely ferment dietary fibers; in Bacteroidota, these are encoded within Polysaccharide Utilization Loci (PULs). Given the rise of inflammatory and metabolic diseases linked to gut dysbiosis, understanding microbial metabolites and dietary factors that influence their production is important. We hypothesized that PULs activated during fiber fermentation in the human gut by *Bacteroides ar*e associated with specific metabolites. Using transcriptomics and metabolomics, we identified PULs involved in the breakdown of pectin, wheat arabinoxylan, and arabinan. We also examined metabolomic profiles of four Bacteroides species during growth on apple and citrus pectin, as well as metabolites produced by B. intestinalis fermenting insoluble wheat and corn arabinoxylan, compared to the monomer sugar units galacturonic acid and xylose, respectively.

PULs activated by different dietary polysaccharides were distinct and substrate-specific, revealing discrete transcriptional genes for pectin, arabinoxylan, and arabinan utilization. Metabolomic analysis identified key metabolites, including SCFAs, amino acids, and organic acids. 26 compounds were prominently produced during pectin fermentation. Notably, gamma-hydroxybutyric acid, inositol, jasmonic acid, and other compounds varied among species and fermentation stages of citrus and apple pectin. In addition, ferulic acid and 4-methylesculetin levels increased significantly during the fermentation of insoluble wheat arabinoxylan and corn arabinoxylan. These findings highlight species-specific metabolic responses and identify potential bioactive compounds with potent health-related benefits including anti-inflammatory, neuroprotective, and immunomodulatory. The identified metabolites produced during the growth of *Bacteroides* spp. on the respective polysaccharides allow us to link each PUL to a metabolite profile.

Quantification of the selective potential of phenylpropanoid monomers on nitrogen-fixing populations of maize-associated bacteria

Trever L. Thurgood, Roland C. Wilhelm

Purdue University

Plant metabolites dynamically modulate tissue-associated bacterial communities and may be involved in mediating responses to environmental conditions. The impact of individual metabolites or classes of metabolites in modulating bacterial community structure through exclusion ("push") or selective promotion ("pull") is not known in all cases. Here, we hypothesize that monomeric derivatives of the phenylpropanoid pathway are implicated in enhancing growth and activity of

beneficial (diazotrophic) bacteria through molecular selection. Through *in vitro* experimentation, we quantify the effects of different individual and combined phenylpropanoid monomers on maize-associated bacterial communities, including diazotrophs. These results point toward potential genetic targets of crops that could be used to enhance nitrogen acquisition through selective manipulation of diazotroph populations.

Dormancy enables viral persistence in soil microbiomes

Joy M. O'Brien, Jitul Bora, Sungyun Kang, Jay T. Lennon

The vast majority of bacteria in soil microbiomes are dormant. By being able to enter a reversible state of reduced metabolic activity, bacteria are able to survive in harsh and fluctuating environments. Endosporulation is a type of dormancy that involves a highly regulated developmental process in which a vegetative cell transitions into an inert spore that is not only protected against extreme environmental conditions, but also virus infection. However, phages have evolved to co-opt endosporulation by becoming entrapped in the developing endospore, forming a virospore. Here, we investigate how viral genetic elements and infection dynamics influence phage entrapment during sporulation and provide evidence for entrapment in nature. We hypothesize that genetic elements aid in the entrapment process and that timing of infection and multiplicity of infection influence entrapment efficiency across stages of spore development. This work provides insight into how phages persist through host dormancy and establishes a framework for understanding potential phage-host interactions and coevolution across microbiomes.

Investigating the paradoxical effect of oral aspiration on the lung microbiome using computational and in-vitro modeling

Bond, K.B., Pickens, C., Sumner, J., & Hartmann, E. M.

Interdisciplinary Biological Sciences (IBiS)/Department of Civil and Environmental Engineering, Northwestern University

Lung microbial communities play critical, but under-characterized roles in health. Bronchoalveolar lavage (BAL) samples from patients with severe pneumonia provide a rare window into lower respiratory tract ecology. Analyses of these samples have identified distinct microbial community states that correlate with patient outcomes. Notably, more oral-like lung microbiomes are associated with improved outcomes, presenting a paradox: aspiration occurs routinely in health yet can also contribute to pneumonia itself. This raises key ecological questions regarding the context by which oral immigration is beneficial or harmful and

whether communities reflect intact transfer or selection-driven restructuring within the lung.

To address this, BAL samples from pneumonia patients were analyzed using amylase as a proxy for salivary input. Samples were stratified by amylase content. Increasing amylase was associated with elevated neutrophil percentages, consistent with controlled immune engagement. A Bayesian mixture model (SourceTracker) estimated that oral contribution to lung communities increased with amylase but remained modest (~35%), supporting a model of seeding followed by environmental filtering. 16S rRNA sequencing revealed no differences in alpha diversity but significant beta diversity shifts, with enrichment of oral-associated taxa. To explore persistence mechanisms, genus-level oxygen tolerance profiles from BacDive were mapped onto BAL communities using relative abundance. Increasing amylase was associated with increased anaerobic and microaerophilic representation. Consistent with the oral cavity, where polymicrobial aggregates support coexistence of aerobic and anaerobic taxa, similar organization may generate localized oxygen gradients that enable anaerobic persistence within the lung.

To further investigate these findings, a controlled bioreactor platform is under development. A 10-member consortium of lung-associated species was cultured under lung-mimicking versus nutrient-rich conditions with dynamic oxygen control. Lung-like conditions enriched *Rothia*, reduced *Streptococcus*, and promoted aggregate formation, while nutrient-rich favored *Corynebacterium* and *Staphylococcus*. Together, these findings support a model in which lung microbial communities are shaped by oral immigration and lung-environment filtering.

Influence of Fusarium graminearum on the bacterial community in Cannabis sativa

Austin Skenandore, Henry Smith, Dr. Nicole A. Gauthier, Dr. Luke A. Moe

Plant and Soil Sciences, University of Kentucky

Fusarium graminearum is a fungal pathogen that mainly affects crops like maize and cereal oats but can also infect other crops such as *Cannabis sativa* (hemp). This pathogen devastates and lowers the overall yield for crops by infecting the head, flowers, and grain of crops. This fungus also can spread a mycotoxin which is harmful to both humans and livestock. These problems lead to *F. graminearum* being one of the most prevalent crop infections in this era and learning about its infectious habits and life cycle is crucial to protecting the food industry. The bacterial community of plants plays a key role in development and health of the host plant. This communication network between microorganisms and hosts is crucial in understanding the role that bacteria have in protecting the host plant.

This study focuses on the impact of infection of *F. graminearum* on the bacterial community present in hemp floral material and we hypothesize that the plant microbiome can serve as a protective barrier to pathogen colonization. Floral material was taken from fifty-three living plants of various cultivars, separated into groups of asymptomatic and symptomatic tissue. 16S rRNA Illumina v2 amplicon sequencing was performed to assess the bacterial communities for each sample. Quantitative PCR was used to determine levels of *F. graminearum* infection in each sample. Sample communities were compared between two groups, symptomatic and asymptomatic. Quantitative PCR results validated the visual assessment of symptomatic/asymptomatic tissue. Cooccurrence network analysis was also performed to judge the difference between community clustering and showed unique community structural differences across groupings. The cooccurrence network analysis preformed supported the hypothesis that plants with more in-tact bacterial communities presented with less *F. graminearum* infection both visually and through relative amount of DNA.

Exploring the impact of the microbiome on bourbon fermentation efficacy and volatile production

Samuelson, Cooper[1]; Heist, Melanie[2]; Barzee, Tyler[3]; Moe, Luke[1]

[1]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY; [2]Ferm Solutions, Danville, KY; [3]Department of Biosystems and Agricultural Engineering, University of Kentucky, Lexington, KY

The unique flavor profiles that distinguish bourbon brands are predominantly formed from variations in mash bills, yeast strains, and maturation practices. Yeast strains generate the foundation of the flavor profile during fermentation. However, fermentation is not a sterile process, as other microorganisms colonize and persist in distilleries despite industrial cleaning procedures. We hypothesize that the collective microbiome within the fermentation ecosystem contributes to the overall success and chemical makeup of the resulting spirit. To determine the impact of bacteria, we inoculated fermentations with species commonly found in distilleries: *Limosilactobacillus fermentum* (formerly *Lactobacillus fermentum*), *Enterococcus faecalis*, *Pediococcus pentosaceus*, and *Acetobacter pasteurianus*. We assessed the impact of single species inoculations at two titers (10^6 and 10^8 CFU/mL) on fermentation efficacy and volatile production. A small-scale, high-throughput fermentation strategy was developed and optimized for consistency across batches, enabling parallel studies between yeast-only fermentation and those with bacterial inocula. A 70/20/10 corn, rye, malted barley grain bill was used to reflect the standard bourbon grain bill. A one-way ANOVA revealed that *L. fermentum, E. faecalis,* and *A. pasteurianus* at 10^8 CFU/mL had significantly lower concentrations of ethanol compared to the yeast-only control, along with further differences that varied both by bacterial species and titer. A two-way ANOVA, used to compare

differences between the inoculated fermentations, showed that volatile production varies the most by species. For instance, isoamyl acetate, an ester with a banana aroma, was found to have the greatest concentration in samples inoculated with *P. pentosaceus*. Additionally, interaction effects were found between species and titer for some fermentation and volatile measurements. These results indicate that bacteria have a significant effect on fermentation and present opportunities for greater refinement of flavor within the alcoholic beverage industry.

Digital enrichment of hyperthermophilic Archaeal viruses from a natural hot spring reveals local diversification in response to host CRISPR-immunity

Thomas W. Cowell, Changyi Zhang, Jiayue Yang, Chih-Lin Chen, Mark Young, Hee-Sun Han, Rachel J. Whitaker

Department of Microbiology, Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign

Capturing the diversity of natural environments is crucial to understanding the evolutionary and ecological processes that shape life on Earth. Despite technological advances, environmental sampling of the strain level diversity of microbial species and their viruses remains a slow and laborious process. Alternatively, metagenomic approaches can recover large numbers of microbial sequences but are limited by incomplete genome assembly and struggle to capture rare but critical community members and link their patterns of covariation. Recent developments in drop microfluidics show potential to improve our understanding of natural systems by enabling the high-throughput processing of individual cells or viruses within complex environmental samples. Here, we extend drop cultivation techniques to the archaeal domain, facilitating highly parallelized enrichment of hyperthermophilic viruses that infect and lyse their archaeal hosts. After developing and validating this approach, we enriched and deeply sequenced viruses collected from a geothermal area within Yellowstone National Park, where the model archaeon *S. islandicus* and its associated virus *S. islandicus* rod-shaped virus (SIRV) interact through lytic infection and CRISPR-mediated immunity. By compartmentalizing the naturally occurring diversity of environmental samples into millions of nearly identical droplets, we eliminate competition between viral strains allowing each unique individual to replicate in parallel on an immune incompetent bait host. This method of viral enrichment outperformed traditional liquid culture enrichment and maintained sufficient depth across many unique viral representatives, revealing the deep local diversity of SIRVs. Analysis of the SNP-level variants identified local viral adaptation in response to host immunity mediated by CRISPR targeting.

Evaluate the ımpact of aldicarb exposure on the gut microbial communities

Jeremiah W. Wanyama and Christopher A. Gaulke

Pathobiology Department, University of Illinois at Urbana-Champaign

Aldicarb is a highly toxic pesticide with neurotoxic effects and links to cancer. While its impacts on the brain have been described, its effects on host microbial community is poorly understood. Gut microbial communities are both sensitive to xenobiotic exposures and highly capable of transforming these compounds. Here, we aimed to define its impacts on gut microbial communities, isolate microbes that exhibited differential sensitivity and resistance, and to identify potential mechanisms that underpin these differences.

To evaluate the impact of aldicarb on gut microbial communities, we exposed 60 adult zebrafish to one of three diets containing aldicarb (0.5, 5, and 50µg/kg) or a control diet. Fecal samples were collected at day 0, 7, and 14 of exposure and microbial communities were profiled using16S rRNA sequencing. Resistance profiles of isolated taxa were characterized using growth kinetics in mineral salts media containing increasing aldicarb concentrations (0.01-1.0mg/ml). Acute exposure was associated with moderate disruption of the gut microbiome. Interestingly, responses to aldicarb were delayed in males and were only apparent at day 14, while female disruption began at day 7. Exposure was associated with increased abundance of several genus whose members can metabolize aldicarb, including *Pseudomonas* and *Rhizobium*. To evaluate if increasing taxa may utilize aldicarb, we isolated dozens of *Pseudomonas* and *Rhizobium* strains from zebrafish fecal microbial communities in selective aldicarb-containing media. Several of the isolated taxa were capable of minimally disrupted growth even at 1mg/ml aldicarb concentrations. Of note, *Pseudomonas* isolates varied substantially in their ability to grow in aldicarb suggesting differential sensitivity of this genus to aldicarb exposure. Our results suggest that zebrafish gut microbiota are both sensitive and resistant to aldicarb exposure, and taxa that increase during exposure may possess machinery to metabolize aldicarb. Ongoing work seeks to identify the mechanistic underpinnings of resistance to aldicarb in specific taxa.

Bee bread mycobiome responses to pesticide exposure in migratory pollination systems

Ling-Hsiu Liao, Wen-Yen Wu, May R. Berenbaum

Department of Entomology, University of Illinois Urbana-Champaign

Colonies of *Apis mellifera*, the western honey bee, involved in delivery of large-scale migratory pollination services typically encounter complex pesticide mixtures across diverse cropping systems, yet effects on the honey bee microbiome under field-realistic conditions remain poorly understood. Microbiome disruption may compromise nutrient acquisition, immune function, and detoxification capacity,

with cascading consequences for pollination services and agroecosystem stability. Because honey bees support crops contributing to roughly one-third of the U.S. food supply, resolving these impacts is critical for colony health and food security. Production of bee bread, a pollen-derived, microbially fermented food that can be stored for later consumption, is integral to maintaining colony health across seasons, during which xenobiotic inputs, fungal activity, and phytochemical processing vary. Disruption of this system may disrupt xenobiotic processing and colony nutrition, with downstream effects on colony health and pollination services. We have characterized the bee bread-associated mycobiome in migratory colonies by integrating pesticide residue profiling with ITS2 amplicon-based community analysis to assess how residue content and composition shape diversity and taxon-specific abundance, accounting for floral source and beekeeping operation. Preliminary results indicate that crop identity and pollination stage are the primary structuring forces influencing the bee bread mycobiome (Permutational Multivariate Analysis of Variance, $p<0.05$), suggesting that floral origin and pollen phytochemical composition across crop types may contribute to structuring bee bread fungal communities. After accounting for these ecological drivers, we found that insecticide and fungicide residues were associated with shifts in fungal community composition. Partial correlation analysis with CLR transformation revealed taxon-specific restructuring rather than uniform diversity loss, suggesting that pesticide exposure selectively reshapes community function with potential consequences for fermentation and colony nutrition. Ongoing metabolomic profiling aims to link these microbiome shifts to altered phytochemical composition, providing a more complete view of how pesticide exposure transforms this complex plant-insect interaction.

Microbial diversity and resistome profiles in suburban waterbodies revealed by long-read metagenomics

Bhuiyan, M.H. & Lee, J.

Division of Environmental Health Sciences, College of Public Health, Ohio State University

Urbanization is rapidly transforming freshwater ecosystems, particularly small suburban waterbodies that are highly exposed to anthropogenic inputs yet remain understudied. These ecosystems receive nutrient runoff, microbial contaminants, and wildlife inputs, resulting in complex environments with potential public health implications. Additionally, rising temperatures due to climate change and intensified land use may further promote microbial proliferation, including the growth of harmful and opportunistic taxa. This study investigated microbial community structure and antibiotic resistomes in two suburban golf course ponds using long-read metagenomics integrated with environmental data. Surface water samples were collected weekly from May to September 2024, and key

physicochemical parameters were measured. DNA was extracted from filtered samples and a subset of 10 samples from different time points across the two ponds was selected for microbiome and resistomes analysis using Oxford Nanopore long-read metagenomics sequencing. The results revealed distinct microbial community compositions between ponds, alongside a shared core microbiome. At the genus level, freshwater-adapted taxa such as *Photobacterium, Streptomyces*, *Staphylococcus* and *Salmonella* were consistently abundant. No significant difference in α-diversity of the microbiome was observed, but the Bray-Curtis distance of β-diversity differed significantly between the two ponds (PERMANOVA, p=0.023). Redundancy analysis indicated that environmental variables strongly influenced microbial structure (adjusted R^2 = 0.77, p = 0.001), with total nitrogen, turbidity, pH, chemical oxygen demand, and UV254 identified as significant drivers. Diverse antibiotic resistance subtypes (e.g., bla, sul1, qac, qacE, qacG, sugE, bacA) were detected in samples, with evidence of association with specific bacterial genera and potential plasmid mediated mobility. These findings demonstrate that suburban waterbodies are dynamic ecosystems shaped by environmental conditions and anthropogenic pressures, capable of harboring and potentially disseminating antibiotic resistance. The study emphasizes the importance of incorporating small urban waterbodies into One Health frameworks to better assess environmental contributions to antimicrobial resistance and public health risk.

Fecal microbes from male mice with liver cancer are sufficient to alter bile acid homeostasis when transferred to females

Lucia M. Thompson[#1], Laura R. Hovenga[#1], François Reichardt[#1], Lauren N. Lucas[2], Angela E. Dean[1], Lydia Okyere[3], Daniel Amador-Noguez[2], Chris A. Gaulke[3], Sayeepriyadarshini Anakk[1]

[#]Equally contributed
[1]Molecular & Integrative Physiology Department, UIUC; Cancer Center at Illinois (CCIL) [2]Department of Bacteriology, University of Wisconsin-Madison
[3]Department of Pathobiology, UIUC

Hepatocellular carcinoma (HCC) incidence in the United States has tripled in the last four decades and is linked to bile acid (BA) homeostasis dysregulation. These cholesterol metabolites produced in the liver are secreted into the gut to help digest fat. In the gut, microbes transform BAs prior to their reabsorption and transport back to the liver via the portal vein. We recently showed that microbes along the gut differ between healthy female and male mice, and these sex differences are reflected in the portal BA pool. Moreover, we also found that sexual dimorphism in the portal BA pool persists in pathological states, as seen in the genetic mouse model of BA overload. This murine model of spontaneous liver cancer (DKO) mimics clinical findings such that the cancer incidence is

predominant only in males at 12-months of age. Male mice exhibit higher levels of 12-OH BAs (generated from cholic acid (CA), and its taurine conjugated form TCA), whereas secondary BAs (e.g., UDCA and DCA) were higher in female portal circulation. Therefore, we examined if sex differences in microbiota were causing this BA alteration. To do this, we performed fecal microbe transplantation (FMT) between the two sexes of DKO mice (i.e. males to females and from females to males) and then re-examined the portal BA composition. Although 16S sequencing confirmed the altered microbiome, 2-week FMT of female-derived microbes did not impact male portal BAs. On the other hand, the transfer of male microbes to DKO females was sufficient to change their BA pool through increases in 12-OH BAs and CA, thus mimicking the male portal levels. Excess CA has been shown to correlate with liver cancer. Ongoing liver histology of DKO females post male-FMT will reveal if portal BA changes can make female livers susceptible to tumorigenesis, highlighting the essential role of gut microbes in promoting HCC.

Dysbiosis fuels bacterial translocation to the pancreas worsening chronic pancreatitis

Sajan C Achi[1], Holly Attebury[2], Dominik Awad[3], Yessine Baalouche[1], Daley Donnele[1]

1 Department of General Surgery, 2 Cancer Biology Graduate Program, 3 Molecular and Integrative Physiology, University of Michigan

Introduction: Chronic pancreatitis (CP) lacks effective therapies, partly due to poorly defined cellular and microbial drivers. Exocrine pancreatic function shapes gut microbial composition, and its progressive loss in CP is often accompanied by dysbiosis. However, the role of dysbiosis in CP progression remains unclear. Here, we investigated the impact of dysbiosis on CP severity and the mechanisms underlying this effect across multiple mouse models.

Methods: Microbial dysbiosis was induced in the Caerulein-induced CP (CICP) and T7D23A CP mouse models by administering an oral antibiotic cocktail. The pancreas, mesenteric lymph nodes, colon and fecal pellets were collected. 16S DNA sequencing was carried out on collected tissue samples to reveal the presence and changes in the microflora. The severity of pancreatitis was determined by pancreas weights, and immunohistochemistry grading tissue loss, acinar cell loss, cell proliferation, and acinar to ductal metaplasia. Bacterial translocation to mesenteric lymph nodes and pancreas was examined by Fluorescence In Situ Hybridization (FISH).

Results: Antibiotics promoted gut dysbiosis in both mouse models (CICP and T7D23A) and resulted in worsened disease. Antibiotics further increased disease severity with progression of age in T7D23A mice. Fecal analysis revealed an increase in pathobionts (*Escherichia*, *Shigella*, *Enterococcus*) and loss of beneficial bacteria (*Bifidobacterium*, *Lactobacillus*) in CP mice treated with antibiotics compared to

CP mice with normal gut microbiome. Bacterial translocation to pancreas and mesenteric lymph nodes were observed in both CP and antibiotic treated CP mice in both the models. Histologic analysis of the colon revealed presence of goblet cell associated passages (GAPS) in dysbiotic mice implicating their role in bacterial translocation.

Conclusion: Our study establishes that dysbiosis in CP increases colonic GAPS and drives bacterial translocation to pancreas, resulting in more severe inflammatory response in the pancreas. Targeting GAPS and bacterial translocation may be a therapeutic option to ameliorate disease severity.

Water management restructures soil microbiomes across agricultural landscapes

Luo, J.P., Jay. T. Lennon

Department of Biology, Indiana University

Global food production is increasingly threatened by shifts in precipitation patterns, including more frequent and severe droughts driven by climate change. These altered hydrological cycles can disrupt nutrient cycling, degrade soil health, and reduce crop yields. Irrigation is the most widely used strategy to mitigate drought stress in agricultural ecosystems. While it often sustains plant growth during dry periods, irrigation may also reshape the structure and function of soil microbiomes, which play a key role in supporting plant productivity and drought resilience. Here, we combined farmer interviews, comparative surveys, manipulative field experiments, and microbial metagenomics across farms in the Corn Belt of the Midwestern United States to identify the causes and consequences of crop irrigation. Farmer decisions to irrigate were influenced by socio-economic factors, including irrigation history and concerns about drought-related loss in crop yield, but were also associated with edaphic characteristics such as soil texture and organic matter content. On unirrigated farms, lower soil moisture and macronutrient availability contributed to reduced plant productivity. Long-term irrigation alleviated these constraints but altered microbial assembly processes, leading to landscape homogenization, the loss of endemic taxa, and the decoupling of plant productivity from functionally important nitrogen-fixing bacteria. Our findings suggest that irrigation reshapes soil microbiomes in ways that alter how belowground biodiversity supports crop productivity, with implications for managing resilient agricultural systems under climate change.

Genomic diversification and tailocin-mediated competition in animal-associated *Xenorhabdus* bacteria

Sarah J Kauffman, Ryan M Awori, Emmanuel C Allwell, Aaron Taylor, Farrah Bashey, and

Heidi Goodrich-Blair

Department of Biology, Indiana University

Biotic interactions, including competition among bacteria mediated by phage-derived weapons, can profoundly shape microbial genomes. We found that compared to genomes across domain *Bacteria*, genomes from the animal-associated *Xenorhabdus* genus contain among the highest proportions of phage-related genes, and variation among strains in their total number of protein-coding genes was largely predicted by variation in total number of non-cargo phage genes per genome. A universal yet highly variable *Xenorhabdus* phage-related locus encoded xenorhabdicin tailocins, a key weapon in bacterial competition. The xenorhabdicin tailocin locus ranged in length from 12 to 41 kilobases and varied markedly within species. Concomitant with this variation, xenorhabdicins produced by six strains of *Xenorhabdus nematophila* differed in killing profiles towards each other. Mutants from two *X. nematophila* strains whose tailocin tail fibre genes were deleted lost their killing ability, while complementation experiments restored or shifted killing profiles, demonstrating that the tailocin locus is responsible for intraspecific killing and the tail fibre gene its specificity. We demonstrated the ecological importance of xenorhabdicin diversity by significantly associating broad differences in intraspecific killing profiles of mitomycin-induced cell lysates from 37 regionally sympatric *Xenorhabdus bovienii* strains with genes within the tailocin locus. The diversity of these genes and their rearrangements within the locus challenges our understanding of tailocin mechanics. We propose that frequent coinfection of insect hosts by multiple *Xenorhabdus* strains promotes strong selection for within-host competitive dominance as well as opportunities for genomic rearrangements, making this genus a valuable resource for examining bacterial evolution in an ecological context.

Effects of high soybean meal inclusion on the gut microbiome of finishing pigs

Angel Martinez, Courtney Pohlen, Joseph Halbur, Eric Weaver, Rafe Royall, Benoit St-Pierre.

Department of Animal Sciences, South Dakota State University

Soybean meal (SBM), the primary protein source in U.S. Midwest swine diets, is typically supplemented with synthetic amino acids to optimize growth. Variation in SBM inclusion can influence pig performance and gut health due to non-protein components, such as fibers that affect microbial metabolism. As new processing facilities expand SBM availability and lower its costs, understanding how high SBM inclusion impacts the gut microbiome is increasingly important. This study aimed to characterize the effects of SBM inclusion levels on swine gut bacterial communities.

546 pigs were used in a 111-day trial to evaluate diets with 1) low SBM inclusion supplemented with synthetic amino acids (5%; LowSBM) vs 2) high SBM inclusion (28%; HighSBM). To determine bacterial composition,14 fecal samples per treatment were collected for DNA extraction and 16S rRNA sequencing through PCR amplification of the V1–V3 regions from microbial DNA. Shotgun metagenomic sequencing on selected samples was performed to assess microbial functional potential. Reads were assembled with MEGAHIT, binned with CONCOCT, MaxBin2, and MetaBAT2, and annotated using DRAM.

Beta diversity by 16S indicated significant differences in bacterial composition between the two treatments (PERMANOVA, $P < 0.05$). We found specific Operational Taxonomic Units that were in higher abundance in the HighSBM ($P < 0.05$). These included Ssd-39, related to Streptococcus alactolyticus (99.5% identity match; 15% vs. 10%); Ssd-1160, related to Treponema brennaborense (88.0% identity match; 2% vs. 0.1%); and Ssd-1254, closely related to Treponema bryantii (97.0% match; 2% vs. 0.03%). Using metagenome-assembled genomes (assessment: >70% completeness; <10% contamination), annotation revealed metabolic capabilities such as polysaccharide degradation, and butyrate production.

HighSBM diets altered gut microbiome composition and metabolic potential. Short-chain fatty acids potential may be driven by bacteria specialized in SBM polysaccharide degradation. These findings indicate that SBM could play a role in shaping microbial activity in swine

---

Cryopreservation of wheat bran arabinoxylan-degrading bacterial communities and assessment of their revival through in vitro fermentation

Miguel Angel Alvarez Gonzales, Stephen R. Lindemann

Department of Food Science, Purdue University

Reliable long-term preservation of complex microbial communities is essential for microbiome research, biobanking, and functional studies that require the reproducible revival of intact consortia. However, how cryoprotective agents (CPAs), vessel microenvironments, and sealing methods affect community viability, composition, and metabolic recovery after multi-year storage at −80 °C remains poorly understood. In this study, we performed a comprehensive, multi-year evaluation of a wheat-bran-arabinoxylan (WB-Ax)-degrading consortium preserved under six CPA formulations: glycerol (20% v/v) without vacuum sealing (CTRL), glycerol with vacuum sealing (GLY), dimethyl sulfoxide (DMSO), erythritol (ERY), and binary mixtures (GLY–DMSO, ERY–DMSO); stored in serum vials sealed with either butyl or natural rubber septa for up to 42 months. The stored bacterial communities were revived by inoculating WB-Ax and then passaged twice. Community structures of stored and revived samples were analyzed using 16S rRNA sequencing, combined with viability assessments via propidium iodide staining and

propidium monoazide treatment. Overall, glycerol-based preservation, employing both non-vacuum (CTRL) and vacuum-sealed (GLY) methods, and its binary mixture with DMSO (GLY–DMSO) reliably supported high post-thaw viability, minimal membrane damage, stable taxonomic profiles, reproducible fermentation dynamics, and sustained acetate-dominant short-chain fatty acid (SCFA) production across various septum types. Dimethyl sulfoxide (DMSO) alone also effectively preserved viability, although it moderated the metabolic rebound observed during cultivation. Conversely, erythritol showed limited effectiveness as a cryoprotective agent (CPA) for complex microbial communities, causing extensive membrane damage and a high proportion of dead cells. However, combining erythritol with DMSO partially alleviated erythritol-associated lethality. Among all CPAs, septum type influenced the community's trajectory, but not its overall direction. Notably, glycerol-based preservation proved robust across both septum types. This study provides a reproducible framework for microbiome cryopreservation, supporting standardized protocols for storage, revival, and functional analysis in microbiome research.

Native Mass Spectrometry Implies a Potential Ligand of Gnavocin, A Narrow-spectrum Bacteriocin Produced by IBD-associated bacteria *Mediterraneibacter gnavus*

Ngoc Pham, Matthew T. Henke

Department of Pharmaceutical Science, University of Illinois Chicago, Chicago, IL, USA

Alterations in gut microbial composition, known as gut dysbiosis, are one of the key factors that drive inflammation in inflammatory bowel disease (IBD). Among human gut microbes, *Mediterraneibacter gnavus* (formerly known as *Ruminococcus gnavus*) abnormally elevates in people with IBD, particularly Crohn’s disease. This elevation of *M. gnavus* has been observed across different cohorts, whereas it remains at low abundance in healthy individuals. Previously, our lab investigated whether *M. gnavus* increases in the gut could be a result of direct competition with other microbes in the gut by secreting antibacterial molecules. We discovered gnavocin, a bacteriocin with a narrow spectrum of antibacterial activity, produced by isolates of *M. gnavus* from people with Crohn’s disease . The gnavocin gene was found exclusively in *M. gnavus* and was significantly higher in IBD patients across Crohn’s disease cohorts in Spain and the United States. This suggested that *M. gnavus* with gnavocin could play a role in exacerbating IBD conditions. Hence, we sought to unravel the mechanism of action (MOA) of gnavocin. We used mass spectrometry and determined the protein sequence of gnavocin, and the intact mass of gnavocin was 12,083 Da. Despite its size, gnavocin retained on a 30 kDa molecular weight cutoff filter, suggesting atypical physical properties. Using native protein mass spectrometry (native MS), we observed a

potential ligand bound to gnavocin, which likely indicates its mode of action and explains its narrow spectrum. Ongoing work will fully elucidate the chemical structure of this ligand. Understanding *M. gnavus* -derived molecules, such as gnavocin, will help clarify how specific M. gnavus strains compete and bloom in the gut, potentially leading to gut dysbiosis in Crohn's disease. Additionally, pinpointing such factors that drive gut dysbiosis could open avenues toward treatment.

Exploring root flavonoids as potential biological denitrification inhibitors in the maize rhizosphere

Anderson, H. A., Juvik, J. A., Kent, A. D.

Department of Crop Sciences, University of Illinois at Urbana-Champaign

Nutrient retention is a key sustainability issue in modern agriculture, which relies on external nutrient application to maintain high yield. Yet, a large proportion of applied nitrogen (N) fertilizer is lost through nitrate leaching and conversion to nitrous oxide (N2O) by soil microbial communities. Biological denitrification inhibition (BDI) is a promising microbiome-associated trait that can mitigate nutrient losses through denitrification. Specifically, root-derived flavonoids, plant secondary metabolites, have been identified as candidate BDI molecules. As an N-intensive crop, maize would benefit from BDI implementation through breeding for enhanced root flavonoid content.

We examined the rhizospheres of two maize inbreds that accumulate flavonoids, including anthocyanins, in their above-ground tissues (Maize Morado and 320O) and a conventional inbred (B73) after eight weeks of growth. Given their above-ground flavonoid content, we hypothesized that Maize Morado and 320O may also accumulate flavonoids in their below-ground tissues, and that these compounds may contribute to BDI activity. We compared denitrification enzyme activity and nitrate (NO3-) concentration in the rhizosphere to assay for BDI activity. Rhizosphere NO3- content was 41% and 38% higher in Maize Morado compared to B73 and 320O, respectively. Maize Morado exhibited a significant reduction in N2O emissions, a measure of denitrification enzyme activity, when compared to 320O; N2O emissions were lower than in B73, though the difference was not statistically significant. Given the observation of pigmented roots during harvest and the enhanced rhizosphere NO3- content, we hypothesize that Maize Morado will exhibit higher root flavonoid content relative to B73 and 320O. Significant differences in N2O emissions and rhizosphere NO3- content between Maize Morado and 320O suggest that the accumulation of flavonoids in above-ground tissues does not uniformly influence below-ground BDI activity. Future research will quantify the difference in root flavonoid content between all three inbreds and expand the survey to include additional flavonoid-rich varieties.

Empirical dynamic modeling reveals time-varying microbial interaction networks in anaerobic digestion

Chao-Jui Chang[1], Chun-Wei Chang[2], Hsiao-Pei Lu[3], Chih-hao Hsieh[4], Jer-Horng Wu[1]

1.Department of Environmental Engineering, National Cheng Kung University, Tainan, Taiwan
2.Institute of Fishery Sciences and Department of Life Science, National Taiwan University, Taipei, Taiwan
3.Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan
4.Institute of Oceanography, National Taiwan University, Taipei, Taiwan

Anaerobic digestion is a microbiome-driven biotechnology for converting organic waste into renewable biogas. Although reactor performance is often interpreted using operating conditions or the abundance of key functional taxa, these factors alone do not fully explain system stability. In anaerobic systems, microbiome function emerges not only from which microorganisms are present, but also from how they interact over time. This is particularly important in anaerobic processes, since the conversion of organic matter to biogas relies on coordinated activities across multiple trophic groups. However, quantitatively characterizing these dynamic interactions in complex bioreactors remains challenging. In this study, we investigated how microbial interaction dynamics change during reactor startup and how these dynamics are associated with reactor stabilization. We reconstructed time-varying microbial interaction networks from daily time-series data collected during startup of a sucrose-fed methane-producing bioreactor. Using a multiview distance-regularized S-map approach within the empirical dynamic modeling framework, we quantified temporal changes in interaction topology. We further examined the causal relationships between microbial interactions, reactor performance, bioenergetics, and community assembly using convergent cross mapping. The results showed that microbial interaction networks were highly dynamic during community succession, even under apparently stable operating conditions. Changes in network topology were closely associated with reactor performance. As the reactor progressed toward a more stable functional state, interaction network stability increased, accompanied by increased interaction strength and complexity. Causal analysis further revealed a feedback loop among bioenergetics, community composition, and microbial interactions, indicating that anaerobic reactor performance is shaped not only by microbial assembly, but also by dynamic interaction processes. This study shows that considering microbial interaction dynamics improves our understanding of ecological processes underlying the stability of performance and succession of microbiomes in anaerobic digestion.

Sinorhizobium fitness on non-leguminous plant hosts

Blaszynski, M., Ahmari, K., Heath, K.

Department of Plant Biology, University of Illinois Urbana-Champaign

Facultative mutualisms are widespread across many ecosystem types and provide important ecosystem services. However, it remains unclear how these mutualisms are maintained over time, as the free-living and partner-associated states may impose distinct, and at times, conflicting selective pressures. It is thus critical to understand how fitness trade-offs between host-associated and free-living periods drive the evolution of mutualists. Using the *Medicago- Sinorhizobium* model system and multiple sympatric non-legume hosts that *Sinorhizobium* is likely to encounter in the environment, I will address if the fitness of *Sinorhizobium* strains change between nodulating (compatible legume) and non-nodulating (other) hosts. If interactions in non-legume rhizospheres influence the relative fitness of *Sinorhizobium* strains, this could have significant implications for the *Medicago-Sinorhizobium* facultative mutualism, especially if low partner quality strains gain a selective advantage in the non-legume rhizosphere. To test if plant-mediated selection varies between legume and non-legume hosts, both *M. truncatula* and six non-leguminous hosts and single or mixed community *Sinorhizobium* strains will be utilized across experiments to understand the evolution of strain partner quality variation. This work is pivotal for understanding how alternative selective pressures shape the evolution of the legume- rhizobia and stability of this interaction.

An integrated gene catalogue of the zebrafish gut microbiome reveals significant homology with mammalian microbiomes

Bushra Fazal Minhas[1], David Alvarado[1], Lydia Okyere[1], Luoyan Duan[1], Jeremiah Wanyama[1], Laura M. Beaver[6], Courtney R. Armour[1], Ian R. Humphreys[1], Carrie L. Barton[7], Michael Kent[5], Robyn L. Tanguay[7], Emily Ho[6,8], Thomas J. Sharptom[5,9], Christopher Gaulke[1,2,3,4]

[1]Department of Pathobiology, University of Illinois Urbana-Champaign, Urbana IL 61802, USA
[2]Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana IL 61802, USA
[3]Personalized Nutrition Initiative, University of Illinois Urbana-Champaign, Urbana IL 61802, USA
[4]Cancer Center at Illinois, University of Illinois Urbana-Champaign, Urbana IL 61802, USA
[5]Department of Microbiology, Oregon State University, Corvallis, OR, 97331, USA
[6]School of Biological and Population Health Sciences, Oregon State University, Corvallis, OR, 97331, USA
[7]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, 97331, USA

[8]Linus Pauling Institute, Oregon State University, Corvallis, OR, 97331, USA
[9]Department of Statistics, Oregon State University, Corvallis, OR, 97331, USA

The gut microbiome is a central regulator of host physiology with well-established roles in modulating metabolic, immune, neuroendocrine and cardiovascular systems. Comprehensive characterization of these host-microbe interactions requires organism specific gene catalogs that provides a foundation resource for taxonomic profiling and functional metagenome analysis. Zebrafish (*Danio rerio*) has been emerging as powerful vertebrate model for gut microbiome research owing to their suitability for high-throughput experimental designs, small laboratory footprint, and cost effectiveness. Despite their growing use, integrated gene catalog (IGC) of zebrafish gut microbiome has been missing – a gap that limit our ability to investigate the underpinnings of host-microbe interactions and understand the genetic and functional diversity of zebrafish gut microbiome. Here we constructed a comprehensive zebrafish fecal metagenomic catalog containing 1.24×10^7 unique full-length proteins derived from 5,593 metagenome assembled genomes using shotgun metagenomic sequences, from four commonly used zebrafish lines housed at five aquatic facilities. We observed novel taxonomic and functional diversity in our IGC resource and found substantial differences in microbiome structure and function across facility, diet and sex. We further utilized this catalog to conduct cross species comparisons among human, fish and mouse microbiomes. Despite the difference in dominant microbial taxa in zebrafish compared to human and mouse, we observed a significant overlap in metagenomic functional capacity suggesting the core gut microbial function are conserved across vertebrates irrespective of taxonomic composition. Our catalog provides a fundamental resource that enable taxonomic and functional investigation of zebrafish gut microbiome and lays ground for cross species comparison of gut microbiome function.

Differences in colonic crypt depth between sire lineages in crossbred pigs

Maya Patel & Dr. Brett Loman

Food Science and Human Nutrition, University of Illinois Urbana-Champaign

Background: The microbiome of livestock plays a critical role in production traits of economic importance, including growth efficiency, nutrient absorption, and overall health. Understanding how microbial communities influence intestinal physiology is an essential step toward identifying functional relationships between the gut microbiome and host biology, with implications for improving livestock productivity and global food systems.

Objective: This study aimed to determine whether genetically diverse pigs exhibit differences in gut microbiome composition and colon morphology.

Methods: Four genetic piglet lineages were generated through artificial insemination using breedspecific semen representing two low-performance and two high-performance breeds (Berkshire, Chester, Landrace, and Duroc) crossed with PIC Camborough sows. A total of 48 piglets (six barrows and six gilts per genetic cross) were included in the study. Colon contents and tissue samples were collected to analyze microbial composition using 16S rRNA gene amplicon sequencing and to assess intestinal morphology. Colon sections were fixed and stained with hematoxylin and eosin (H&E) for measurement of crypt depth. Data were analyzed using two-way ANOVA, with significance set at $p < 0.05$.

Results: Gilts exhibited greater microbiome diversity compared to barrows ($p = 0.02$), based on total amplicon sequence variants (ASVs). Additionally, microbial community composition differed by breed, with Chester and Yorkshire pigs showing significant differences compared to Duroc ($p = 0.032$); Unweighted UniFrac). Morphologically, Yorkshire pigs demonstrated increased crypt depth relative to Duroc and Berkshire pigs, though not significantly different from Chester ($p = 0.017$).

Conclusion: These findings suggest that breed-specific genetic variation influences both gut microbiome composition and colon morphology in pigs. This work provides a foundation for further research linking host genetics, microbial ecology, and intestinal function, with potential applications in improving livestock health, productivity, and food security.

Comparative biofilm growth on microplastic surfaces in drinking water: Polypropylene, polystyrene, and cellulose acetate

Haque, A.[1], Proctor, C. R.[1,2]

[1]Sustainability Engineering and Environmental Engineering, Purdue University
[2]Agricultural and Biological Engineering, Purdue University

Microplastics, polymers less than 5 um in diameter, are commonly found in drinking water. In addition to their own inherent health risks, they can act as surfaces where microorganisms attach and form biofilms. However, different types of plastics may support biofilm growth differently. In this study, we examined how three common microplastics- polypropylene (PP), polystyrene (PS), and cellulose acetate (CA) affect biofilm formation under conditions similar to drinking water systems.

We incubated the microplastics in conditions mimicking low-nutrient drinking water conditions. Over time, we measured both water and microplastic surfaces to track microbial growth. Flow cytometry was used to measure total and intact cell counts in both the water matrix and on the microplastics. The results showed clear differences between the materials. Cellulose acetate had the most biofilm growth,

followed by polystyrene and then polypropylene. These differences are likely due to variations in plastic properties such as chemical composition, which influence nutrient leaching and microbial attachment.

We also studied the effect of weathering by comparing weathered and unweathered microplastics. Scanning electron microscopy (SEM) showed changes in surface structure after weathering. Confocal laser scanning microscopy (CLSM) was used to observe cell growth and biofilm structure, and these results were consistent with the flow cytometry data.

Overall, this study shows that both the type of plastic and its condition affect biofilm formation in drinking water. Materials like cellulose acetate may support more microbial growth where polystyrene and polypropylene may almost represent inert condition. Findings of this study aim to help understand the risks of microplastics in drinking water systems.

Metagenomic analysis of wastewater treatment impact on microbial plastic degradation potential in the Chicago area waterways

Megan Lucas, Rachel Poretsky

Department of Biological Sciences, University of Illinois Chicago

Wastewater reclamation plant (WWRP) effluent serves as the primary mode of microplastic introduction into freshwater systems. Microbial communities are known to harbor the capacity to degrade polymers, yet the extent to which this occurs in natural systems remains poorly understood. This study investigates how microbial plastic metabolism is impacted by the wastewater treatment process through a year-long metagenomic sampling effort across three major WWRPs in the Chicago metropolitan area. The three selected WWRPs employ distinct disinfection steps: chlorination, UV sterilization, and no disinfection, enabling a direct comparison of treatment effects on microbial community function.

Water and sediment samples were collected bimonthly at sites upstream and downstream of each plant's effluent discharge, along with samples of raw influent and treated effluent. The Chicago River serves as a particularly informative site for this study, as treated wastewater accounts for approximately 70% of the total river volume. Metagenomic sequencing data were analyzed using the Plastic-Microbial BioRemediation Database to extract genetic and enzymatic markers associated with polymer degradation pathways. This approach allows for the quantification of the microbial plastic degradation potential across sample types, sites, and time points without the constraints of single-species or culture-based methods.

Preliminary results indicate that influent samples show the highest abundance of plastic-associated pathways, with a significant reduction observed in river samples and treated effluent. These findings suggest that the water treatment process

substantially diminishes the plastic degradation potential of associated microbial communities, laying the groundwork for the development of future remediation strategies.

Establishing in vivo and in vitro approaches to investigate urobiome metabolism of environmental chemicals and bladder cancer development

Audra L. Crouch, David Jegede, Brandon McKenna, Madison Griffin, Sheryl Justice, Ahmad El Hellani, Vanessa L. Hale

Department of Veterinary Preventive Medicine, Ohio State University

Interactions between host cells, environmental chemicals, and microbes play a critical role in bladder health and bladder diseases, including bladder cancer (BC). Polycyclic aromatic hydrocarbons (PAHs) are environmental chemicals linked to bladder cancer that are a byproduct of incomplete combustion and are prevalent in charred foods, automobile exhaust, and cigarette smoke, with diet and tobacco smoke serving as primary exposure routes. Benzo[a]pyrene, a prototypical PAH, can be metabolized into secondary metabolites that induce pro-inflammatory, mutagenic, and carcinogenic responses in host tissues. While human cells are known to metabolize BaP, it remains unclear whether bladder-associated microbes can metabolize BaP into reactive intermediates and contribute to inflammatory, mutagenic, or carcinogenic effects in bladder tissues. Importantly, previous studies have shown compositional differences between the urinary microbiome of healthy individuals and those with BC, suggesting potential microbial contributions to disease pathogenesis. Finally, it remains unknown if urinary microbiomes remain stable or dysbiotic after long-term exposures to BaP. To address these gaps, we combined ***in vivo* and *in vitro* approaches** to investigate microbial contributions to BaP metabolism and its impact on bladder health. *In vivo*, mice (N = 36) were gavaged daily with BaP for four weeks, and fecal and urine samples were collected weekly for targeted metabolomics, 16S rRNA amplicon sequencing, and metagenomic sequencing. In a parallel *in vitro* study, a bladder-associated microbial isolate *Pseudomonas aeruginosa* was **co-cultured in the presence of but sans contact with urothelial cells (HBLAK) in a trans-well system, enabling BaP diffusion between compartments**. HBLAK cells exposed to BaP with or without *Pseudomonas* showed increased cytotoxicity in a dose-dependent manner. Cytotoxicity of HBLAK cells increased from 5.5% with BaP alone to 17% when BaP was combined with *Pseudomonas aeruginosa*. Spent media will be analyzed for BaP and its metabolites via targeted metabolomics. In the *in vivo* mouse study, we anticipate distinct microbial community differences between treatment groups, characterized by an increased abundance of taxa involved in BaP and secondary metabolite metabolism. This methodological framework enables controlled assessment of host-microbe-chemical interactions and PAH metabolism across

biological system, providing a foundation for elucidating microbial contributions to bladder cancer development.

Environment and host ıdentity structure root-associated fungal communities of Panamanian Podocarpus spp., revealing helotiales dominance

Martin, B.D.[1], Ferrer, A.[1], Lasso, E.[2,3], & Dalling, J.W.[1,2]

[1]Department of Plant Biology, University of Illinois Urbana-Champaign
[2]Smithsonian Tropical Research Institute
[3]Coiba AIP

Arbuscular mycorrhizal fungi (AMF) are widely assumed to dominate root symbioses in tropical forests; yet, their nutrient acquisition relies heavily on indirect pathways through the soil microbiome rather than direct enzymatic decomposition. On highly weathered, acidic soils where microbial activity is constrained, fungi with broader saprotrophic enzymatic capacity may hold an advantage. The tropical conifer *Podocarpus* persists on impoverished Neotropical soils with unique nodulated roots colonized by AMF. This offers a compelling system to examine which fungal guilds actually dominate under these conditions.

We surveyed root-associated fungi across six Panamanian sites spanning 177–2,440 m elevation, combining ITS and targeted AMF amplicon sequencing across *Podocarpus* and co-occurring vegetation. Soils were acidic and phosphorus-poor but chemically distinct, with site-level differences driven by variation in aluminum, base cations, organic carbon, and nitrogen. This reflects divergent pedogenic pathways along an elevation gradient, providing meaningful environmental contrast for community analyses.

Leotiomycetes, particularly Helotiales, were the dominant fungal clade across all sites and hosts regardless of these soil differences. Within this shared dominance, host identity drove consistent differences in relative abundance: *Podocarpus* roots were enriched in Leotiomycetes and Eurotiomycetes, while co-occurring non-podocarps were enriched in Agaricomycetes and Sordariomycetes. AMF were rare across all hosts; targeted sequencing revealed host-linked enrichment with *Acaulospora* in podocarp roots and *Glomus* in non-podocarps.

These results establish Leotiomycetes as a dominant root-associated fungal clade in nutrient-poor tropical forests, with host identity shaping consistent enrichment patterns across divergent soil environments, pointing to an understudied functional guild in tropical forest nutrient cycling.

A standardized toolkit for programmable protein secretion and surface display in Bacteroides thetaiotaomicron

Yu-Hsuan Yeh and Shannon J. Sirk

Department of Bioengineering, University of Illinois Urbana-Champaign

Bacteroides thetaiotaomicron (*Bt*), one of the most dominant bacterial species in the human gut, is a promising chassis for engineering in situ therapeutic delivery systems. Previously, we developed a secretion toolkit composed of endogenous lipoprotein signal peptides (SPs) and full-length secretory proteins from *Bt*. However, due to variations in length, structure, and amino-acid sequence, these SPs exhibited variable secretion efficiencies across different cargo proteins. Because the activity of different SP-cargo pairs is not readily predictable, screening and optimization is often required to achieve target secretion titers. To standardize this approach and enable predictable and tunable protein secretion, we first identified the lipoprotein export sequence (LES) as the key determinant of efficient secretion of heterologous proteins by lipoprotein SPs. We next performed mutagenesis on the LES region of a representative lipoprotein SP to generate a pool of mutants featuring a standardized SP backbone with diversified LES. Screening and characterization of this mutant pool revealed a charge-dependent regulation of both secretion and surface display of heterologous cargo proteins. With improved tunability, enhanced predictability, and surface display capabilities, this toolkit minimizes the need for iterative screening when developing protein secreting gene circuits for *Bt* and other *Bacteroides* species, and expands the scope of therapeutic applications, particularly those requiring tunable dosing or surface presentation of proteins.

Using HiC metagenomics to characterize the diversity of the rhizobial mobilome between clover rhizosphere and nodules

Bedwell S.L.B[1,2], Heath K.D.H[2,3]

Dept of Microbiology; Carl R Woese Institute for Genomic Biology, Dept of Plant Biology, University of Illinois Urbana-Champaign

It is vital to understand the complexity of microbial systems, and how they shift in response to environmental contexts, to buffer ecosystems against large-scale environmental change. Microbes' interactions with each other, and with eukaryotic hosts like plants, often depend on accessory genes carried on mobile genetic elements (MGEs) such as plasmids and viruses; thus, MGE variation can directly influence ecosystem processes relevant to agriculture and food security. Yet, traditional approaches can't capture this diversity: isolation misses rare but important taxa, chromosomal amplicons miss MGE diversity, and standard metagenomics can't resolve which MGEs belong to which microbial cells. Additionally, co-existing microbes can be chromosomally similar while carrying distinct MGEs. In complex communities where MGEs are gained and lost within a

single host generation, a holistic picture of co-occurring diversity is essential to understanding ecosystem response to change.

The legume-rhizobium symbiosis provides an ideal model to investigate how microbial diversity, both chromosomal and MGE, changes across ecological contexts. Nitrogen fixation by rhizobia, critical in wild and agricultural systems, is a plasmid-encoded trait. Previous studies using amplicon and isolation methods have found immense co-existing rhizobial diversity, chromosomal and plasmid, which is strongly affected by a key environmental shift: long-term nitrogen addition. Here, we leverage HiC long-read metagenomics to examine how the rhizobial mobilome changes under long-term N-addition, and between nodule and soil environments. With this method, we directly link MGEs to their host genomes *in situ,* allowing us to examine not only how the mobilome varies among abundant, closely related strains, but to characterize novel diversity of rare taxa.

By capturing how MGE and chromosomal variation is organized within a key agricultural system, we establish a foundation for understanding how shifts in microbial genome architecture at different scales underpins how communities respond to environmental change– influencing plant health, nitrogen fixation, and the sustainability of food production systems.

Metagenomic characterization of microbial communities in artificial turf fields across Chicago

Malas J.[1], Kaiser J.[1], Sargis R.[2], Hampton-Marcell J.[1]

1. Department of Biological Sciences, University of Illinois Chicago
2. Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Illinois Chicago

Artificial turf (AT) is widely deployed in outdoor green spaces and increasingly promoted as a sustainable alternative to natural turfgrass. However, mounting evidence that AT harbors potentially harmful microbial communities and releases chemical contaminants has raised significant environmental and public health concerns. Among these contaminants, AT leaches a range of toxicants — including both essential and non-essential metals — yet the relationships between metal accumulation and microbial community structure and function in AT fields remain poorly characterized. Of particular concern, AT may serve as a reservoir for antimicrobial resistance (AMR) proliferation through co-selection of microbial and metal resistance mechanisms. Zinc, for example, is consistently detected at elevated concentrations in AT and is well established as a driver of AMR proliferation. To address these knowledge gaps, we characterized microbiome profiles across AT fields throughout the City of Chicago to quantify spatial

heterogeneity and assess whether neighborhood-level pollution is associated with elevated metal accumulation. We leveraged the Air Quality Health Index (AQHI) to select ten athletic fields spanning a wide range of pollution burden, sampling each field in triplicate (n = 30), on the premise that fields in higher-exposure environments may accumulate greater metal loads. Microbial communities and were profiled using long-read metagenomic sequencing on a Nanopore MinION device. Our results reveal a large proportion of methylotrophic bacteria alongside potentially pathogenic species, including *Enterococcus faecalis*. Collectively, this work provides foundational evidence on microbial dynamics in artificial green spaces, with implications for AMR surveillance strategies and urban environmental management.

Exploring how a host-specific microbe modulates early gut immunity in chickens through targeted Kinome Array testing.

Caleb J. Skow & Melha Mellata

Interdepartmental Microbiology Program & Food Science and Human Nutrition, Iowa State University of Science and Technology

Segmented filamentous bacteria (SFB) are a host-specific gut microbes that are important in the early immune development across a wide range of species. Research on mono-colonized mice has indicated a critical role of SFB in programing Th17 cells in the gut and elevating intestinal levels of IgA. Understanding of specific molecular pathway interactions between SFB and its host is limited, especially in chickens. Our lab has demonstrated in chickens that SFB reduce the shedding of *Enterobacteriaceae* in the feces of broilers, enhance bactericidal capability of small-intestinal scrapings and blood serum, decrease gut permeability, and modulate cytokine profiles indicative of localized T-cell activation. This study aimed to further elucidate the interactions occurring in the ileal environment where SFB adhere, using high-throughput assessment of kinome-wide activation states. Conventional Lohmann Select Layers were administered ~10^4 gene copies of SFB-enriched inoculum or PBS at day of hatch. At 15 and 29 days post inoculation, chicks (n=12/group) were euthanized and ileal tissues were collected for analysis via peptide kinome array. Several proteins implicated in non-canonical NF-κB signaling increased phosphorylation at either timepoint in the SFB group. This included the NF-κB p50 and p100 subunits, as well as NFATc3, which inhibits mTOR activity and increases production of MUC2. The dephosphorylation of IκBα in conjunction with the increased phosphorylation of IκBKα indicates that activation of non-canonical NF-κB is responsible for these phosphorylation events. Stimulation of this pathway has various effects, including increased resistance to inflammatory gut pathogens and a critical role in the maintenance of B-cell and the generation of effector memory T-cells. Through enhancing our understanding of the pathways affected by SFB,

we can more strategically employ it to improve host health instead of resorting to more controversial treatments like antibiotic usage.