Overview of MAG Research

DAVID HUYBEN

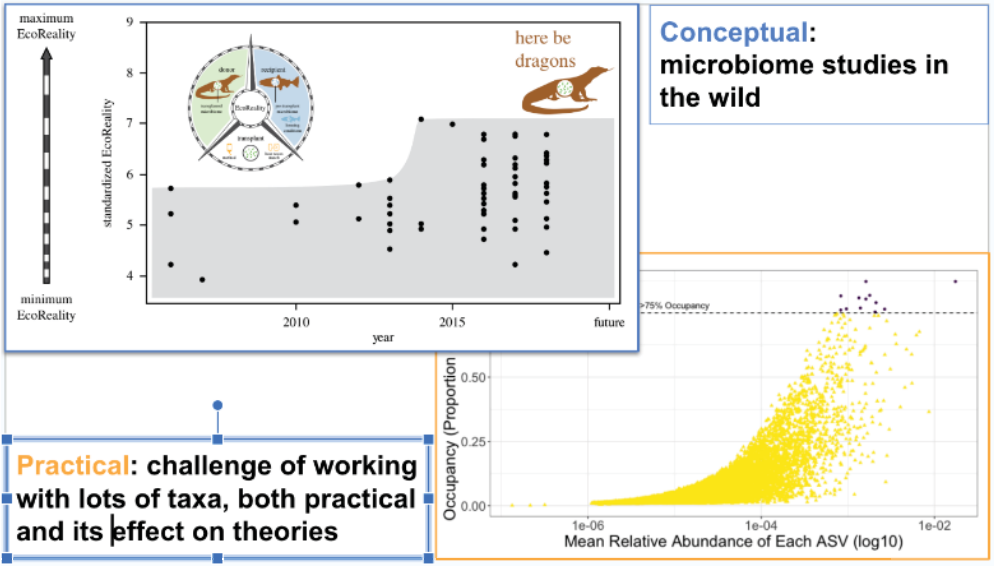
Animal Biosciences, OAC

- Research goal
  - Impact of host and environmental (e.g. diet) on gut microbiome of farmed and wild fishes (trout & salmon), as well as microbial contribution to health and nutrition of fish
- Methods
  - 16S rRNA gene amplicon sequencing (V3-V4) on Illumina MiSeq (AAC-GF/Dalhousie)
  - RNA-seq of metabolic/immune genes on Illumina Novaseq 6000 (AAC-GF/McMaster)
- Vexing question(s)
  - Future methods: can we do low density SNP chip to correlate traits with microbiomes?
  - Future methods: can we do shot-gun sequencing to correlate microbes & function?
  - 16S sequencing: could we use fusion primers for 1 step PCR or go PCR free?
  - Cost: how to reduce library and sequencing costs at UoG?
  - Bioinformatics: where can we send students for training and help?
  - Is anyone else interested in this area or have the same problem?

KARL COTTENIE

Integrative Biology, CBS

- Research goal
  - Applying ecological theories to microbiomes of host populations in the wild
- Methods
  - Analyzing existing data sets
  - Multivariate statistics
- Vexing question(s)
  - The relative importance of dispersal (horizontal migration, transmission) vs. within-host environmental conditions on determining community composition



Conceptual: microbiome studies in the wild

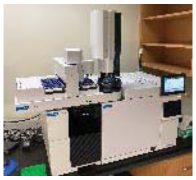
Practical: challenge of working with lots of taxa, both practical and its effect on theories

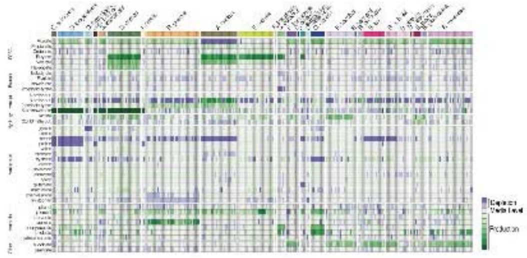
MATTHEW SORBARA

Molecular & Cellular Biology, CBS

- Research goal
  - Take a mechanistic approach to understanding how genomic diversity influences the contribution of individual isolates to colonization resistance and immunomodulatory functions
- Methods
  - Anaerobic culture of gut microbes
  - Bioinformatic analyses
  - Metabolomic profiling by GC-MS
  - Mouse models
- Vexing question(s)
  - Can we harness intra-species, strain level diversity to design better live-biotherapeutics

Marissa → Developing genomic tools in gut microbes
Loudon → Strain-level carbon source utilization
Bradley → Genomic diversity and response to gut stress
Isaac → Role of urease in colonization resistance
Ethel → Variation in immunomodulatory activities across butyrate-producing microbes



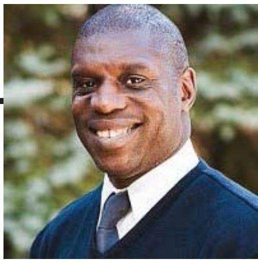


GISELE LAPOINTE

Food Science, OAC

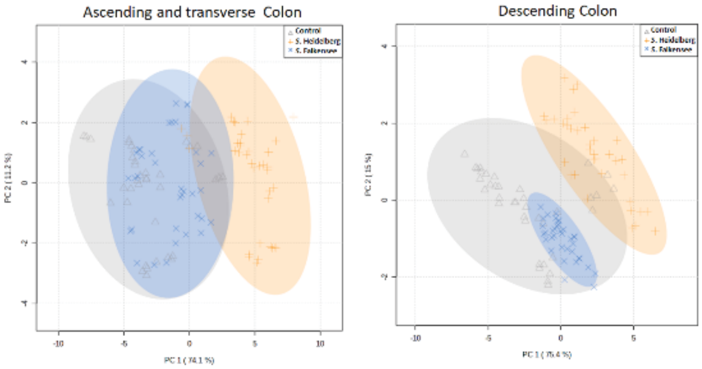
- Research goal
  - Systems approach to food quality and safety (farm to gut)
  - Spoilage and food pathogen survival
  - Impact of food components and structure on gut ecology
- Methods
  - SHIME: *in vitro* models of gut microbial ecosystems (human and animal)
  - 16S rRNA gene and ITS amplicon sequencing (fecal, food and feed such as silage)
  - Shotgun sequencing (milk, cheese and yogurt)
  - WGS of isolates
  - NMR; thanks to Emma's team!
  - SPME-GC-MS
  - Data integration methods (machine learning algorithms)
- Vexing question(s)
  - Samples with fragile cells and low mRNA in complex matrices
  - Databases with disparate taxonomies (fast-paced nomenclature changes)
  - Live/Dead resolution
  - I could go on!

LARRY GOODRIDGE



Food Science, OAC, Canadian Research Inst. for Food Safety

- Research goal
  - Investigate interactions between foodborne bacterial pathogens (*Salmonella enterica*) and the human gut microbiome
- Methods
  - Multiple models of infection (murine, amoeba, cell assays, 3D organoids) are used to assess virulence in *S. enterica* isolates
  - Selected isolates (different virulence levels) are introduced to the SHIME
  - Changes to the microbiome and the *Salmonella* isolates are assessed
- Vexing question(s)
  - How do differences in virulence levels in *S. enterica* affect the human gut microbiome?
  - Does prophage induction during infection "program" the gut microbiome?



S. Heidelberg and *S*. Falkensee variation in metabolic profiles

ZENY FENG

Maths & Stats, CEPS

- Research goal
  - Understand how microorganisms live, interact together as a whole to shape their inhabited environment including: human/animal body, soil, plant, plant, and so on.
  - Understand how microorganisms respond and are impacted by the host.
- Methods
  - Develop and apply statistical and computational tools to analyze metagenomic
  - microbiome sequencing and compositional taxonomic data.
- Vexing question(s)
  - The change of taxonomic classification and functional annotation of sequencing data
  - Quality, accuracy and reproductivity of the sequencing data
  - Dimensionality of the data and interpretation of the analysis results
  - Rarefaction and sparsity of the data

MARY RUTH MCDONALD

Plant Agriculture, OAC

- Research goal
  - Determine if there is a relationship between the soil microbiome and:
    a) soil health of high organic matter soils,
    b) disease risk – cavity spot of carrot
    c) disease risk – clubroot of canola and related crops
- Methods
  - Soil sampling in growers' fields (2–4 sites) and in replicated field trials.
  - Assess yield and disease severity.
  - Soil to labs for general analysis, soil health analysis.
  - Microbiome assessed by Harvest Genomics.
- Vexing question(s)
  - How much does the microbiome change from place to place and year to year?
  - Will any of this information be useful for determining soil health or risk of disease development?
  - How to pay for the expensive microbiome analysis

Soil health and yield

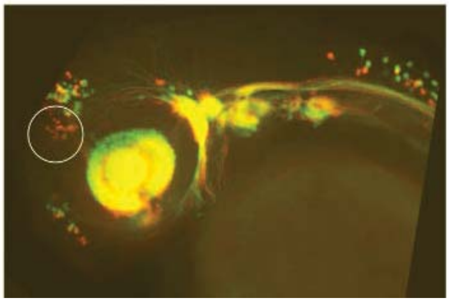
Cavity spot of carrot

Clubroot on cabbage

TERRY VAN RAAY

Molecular & Cellular Biology, CBS

- Research goal
  - Understand the contributions of gut derived metabolites to nervous system development and disorders
- Methods
  - Raw fecal metabolites or Robogut derived metabolites and early zebrafish development
- Vexing question(s)
  - What systems (e.g., limbic) are most sensitive to metabolites and what assays are best suited to address these questions?
  - Is the zebrafish nervous system a good proxy for this line of investigation?



Composite of transgenic zebrafish embryos treated with fecal-derived metabolites from neurotypical children (green) or children with ASD (red) showing differences in location of a sensory neuron marker isl2b.

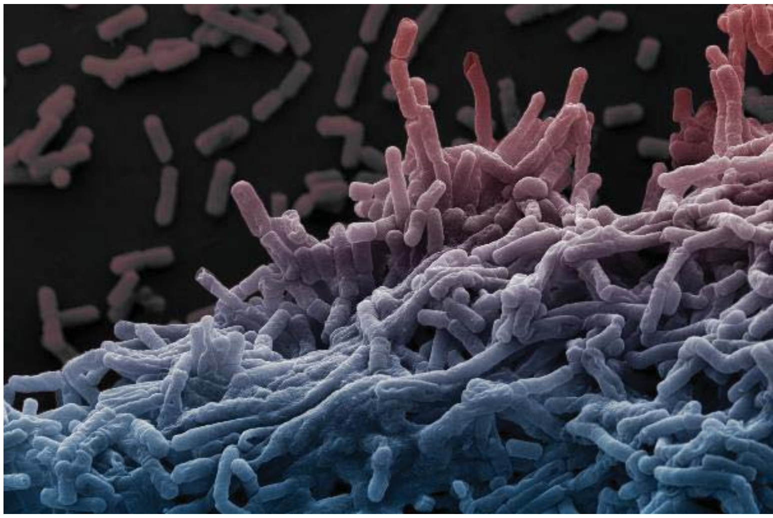
ERNESTO GUZMAN

Environmental Sciences, OAC

- Research goal
    - To better understand how the microbiome of honey bees is affected by biotic and abiotic stressors and how it may impact bee health
- Methods
    - Honey bee tissue extraction and 16 S sequencing
- Vexing question(s)
    - How microbiome changes over time
    - Role of parasites, diseases and pesticides in honey bee decline

CEZAR KHURSIGARA

Molecular & Cellular Biology, CBS

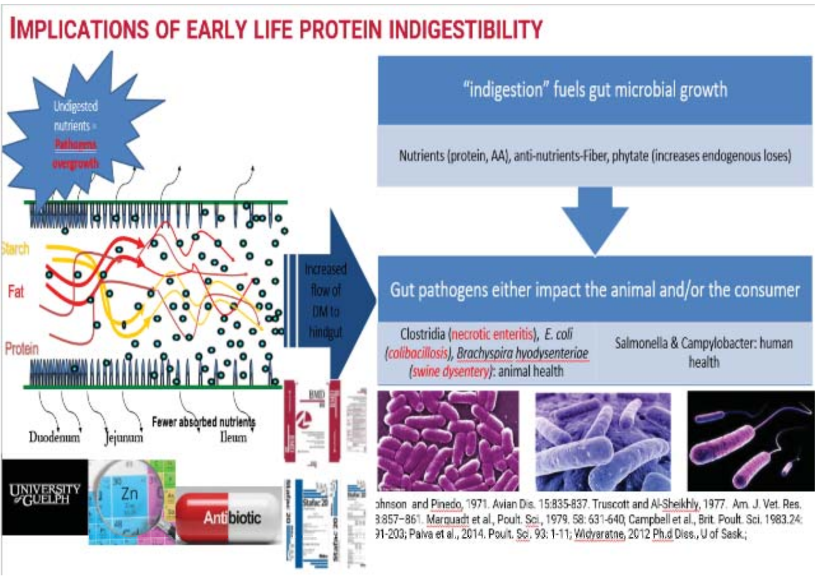


- Research goal
  - Combat AMR by understanding molecular mechanisms related to cell envelope and bacterial biofilms
- Methods
  - Imaging, biochemistry, proteomics
- Vexing question(s)
  - Can we use the ‘microbiome’ to combat AMR

ELIJAH KIARIE

Animal Biosciences, OAC

- Research goal
  - Interaction between gut microbiota and nutrition? How does microbiota influence gut function and health and, subsequently, feed conversion ratio, and growth?
- Methods
  - Relevant methods that can give quantitative data of who is there and what is he doing?
- Vexing question(s)
  - Consistent methods, easy to use in research and farm settings



IMPLICATIONS OF EARLY LIFE PROTEIN INDIGESTIBILITY

"indigestion" fuels gut microbial growth

Nutrients (protein, AA), anti-nutrients-Fiber, phytate (increases endogenous loses)

Gut pathogens either impact the animal and/or the consumer

Clostridia (necrotic enteritis), *E. coli* (colibacillosis), *Brachyspira hyodysenteriae* (swine dysentery): animal health

Salmonella & Campylobacter: human health

ohnson and Pinedo, 1971. Avian Dis. 15:835-837. Truscott and Al-Sheikhly, 1977. Am. J. Vet. Res. 3:857–861. Marquadt et al., Poult. Sci., 1979. 58: 631-640; Campbell et al., Brit. Poult. Sci. 1983.24: 91-203; Paiva et al., 2014. Poult. Sci. 93: 1-11; Widyaratne, 2012 Ph.d Diss., U of Sask.;

VAHAB FARZAN[1,2] & BRANDON LILLIE[2]

Population Medicine[1] and Pathobiology[2], OVC

- Research goal
  - Identify bacterial community members associated with foodborne pathogens, production-limiting diseases, and productivity in swine, as well as genetic markers in swine associated with production, growth, health, and different microbiome populations.
- Methods
  - Observational herd studies and control challenge trials (ex. *Salmonella*, enterotoxigenic *E. coli*, *Streptococcus suis*)
  - 16s rRNA sequencing and amplicon sequencing variant (ASV)
  - Compositional analysis
  - High-throughput genotyping of host (pig) DNA
- Vexing question(s)
  - What are the most significant micro and macro predictors for a “healthy” microbiota composition in swine, and how do host genetics contribute to the development of that “healthy” microbiota?

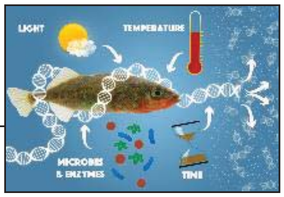
ROBERT HANNER

Integrative Biology, CBS

- Research goal
  - Improve Environmental Monitoring: Baseline Biomonitoring, Environmental Effects Monitoring, Restoration Ecology, Invasive Species/Species-at-Risk
  - Understand Food Webs
  - Food Authenticity and Food Safety (seafood microbiome)

- Methods
  - High-Throughput Marker Gene Sequencing (eDNA metabarcoding)
  - qPCR, ddPCR
  - Bioinformatics (esp for very large data sets)

- Vexing question(s)
  - Ecology of eDNA (origin, state, transport, fate, viability)
  - Estimating abundance from eDNA data
  - Inferring function from community composition
  - Data archival and management



PAUL GOODWIN

Environmental Sciences, OAC

- Research goal
  - To understand how the rhizosphere, endosphere microbiome changes with ginseng replant disease.
- Methods
  - DNA extraction from soil attached to roots and ginseng tissues followed by 16S sequencing.
- Vexing question(s)
  - The cause and prevention of ginseng replant disease.

EMMA ALLEN-VERCOE

Molecular & Cellular Biology, CBS

- Research goal
  - Understanding gut microbial ecology through the lens of in vitro culture
  - Discovery and characterization of ‘missing microbes’
  - Human and honey bee gut microbiome modeling
- Methods
  - *In vitro* continuous complex culture (Robogut and RoBeeGut)
  - Anaerobic bacterial axenic culture/culturomics
  - gDNA sequencing (metataxonomics and metagenomics)
  - Tissue culture, microbial exposure and transcriptomics
  - Metabolomics (1D ^{1}H NMR)
- Vexing question(s)
  - How best to catalogue and share microbial isolates?
  - How to reliably isolate fungal and archaeal species from complex communities?
  - How to transfer microbial ecosystems and components effectively from host to host?

KIERAN O'DOHERTY

Psychology, CSAHS

- Research goal
  - Develop guidelines, principles, metrics for *microbiome stewardship*
- Methods
  - Stakeholder engagement; expert workshops; conceptual analysis
- Vexing question(s)
  - How can we get government, industry, society to adopt policies to protect health microbial environments?

ANGELA CÁNOVAS

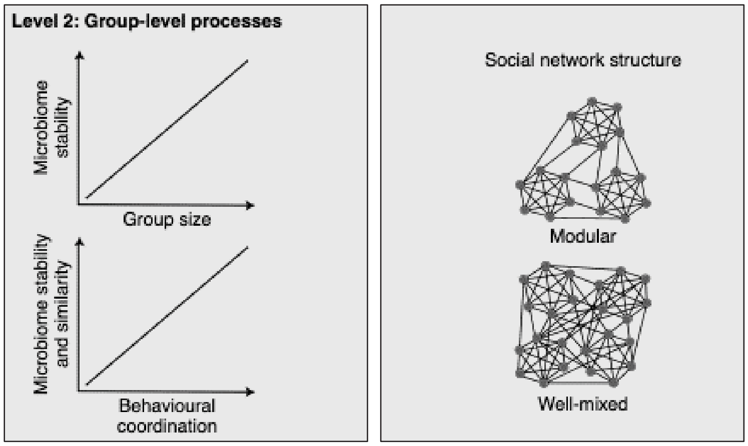
Animal Biosciences, OAC

- Research goal
  - Identify genetic interactions between host transcriptome and rumen microbiome influencing desirable traits in cattle
- Methods
  - Rumen fluid bacteria and archaea 16s rRNA Amplicon Sequencing
- Vexing question(s)
  - What heritable rumen microbiome features can be used to incorporate into genomic selection models for more feed efficient and low methane producing cattle?

QUINN WEBBER

Integrative Biology, CBS

- Research goal
  - Bats highly social and transmission of parasites typically a function of sociality
  - Identify “social microbiome” in bats
- Methods
  - Working towards establishing long-term study on bats in Ontario
- Vexing question(s)
  - Do bats have a social microbiome?
  - How are social networks related to stability/similarity in the social microbiome?



Adapted from Sarkar et al. 2020 Nat Eco Evo

MARC HABASH

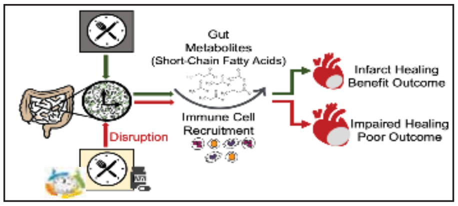
Environmental Sciences, OAC

- Research goal
  - Examining pathogens and fecal indicator microorganisms from natural and man-made environments; including surface and ground waters and wastewater-based epidemiology.
- Methods
  - Molecular and culture-based detection of bacterial and viral pathogens:
  - Molecular/genomic methods: qPCR, dPCR, long read sequencing
  - Cell-culture: cell lines/enteric viruses, bacterial cell culture
- Vexing question(s)
  - Inability of molecular and sequencing technologies to differentiate infectious from non-infectious pathogens (viruses in particular) in environmental matrices
  - Challenges in developing normalization methods using human fecal biomarkers for tracking pathogens in wastewater surveillance (and other matrices)

TAMI MARTINO

Biomedical Sciences, OVC

- Research goal
  - To determine how the microbiome can benefit healing from cardiovascular disease
- Methods
  - Microbiota Disruption
  - Microbiota Reconstitution
  - Circadian Influence & Time Restricted Feeding (TRF) – *“when” and not just “what” you eat is important*
- Vexing question(s)
  - In the future, will hospitals do microbiome reconstitution to improve healing from heart attacks?
  - Can they refine this to create “Heart Attack” yogurts to improve healing of patients
  - Could we make this into a microbiota pill formulation – “heart attack” probiotic pills?
  - Meantime can we use TRF to benefit microbiome & cardiac repair?



JACKIE GOORDIAL

Environmental Sciences, OAC

- Research goal
  - Understand the microbial ecology of organisms in extreme environments
- Methods
  - Amplicon, Metagenomic, Transcriptomic, and single cell sequencing.
  - Cultivation, incubations.
- Vexing question(s)
  - What are the limit of microbial activity in extreme environments?
  - How do slowly growing organisms affect global biogeochemical cycling?
  - How do microbes on Earth inform our search for life elsewhere?

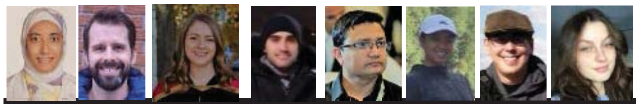
AMY NEWMAN

Integrative Biology, CBS

- Research goal
  - Long term impacts of environmental stress on wild animals
  - How does landscape modification affect the gut-brain axis
- Methods
  - Ecophysiology (hormones, neurophysiology, behaviour, life-history)
  - Field studies
  - 16S
  - One Health
- Vexing question(s)
  - Circularity of the gut-brain axis…what's the direction of causation?!
  - How do we get the sequencing done locally and in a cost-competitive manner?

NEWMAN LAB

ECOPHYSIOLOGY OF STRESS



MANISH RAIZADA

Plant Agriculture, OAC

- Research goal
  - Fundamental: To understand paternal inheritance of the microbiome in plants using corn as a model system. *Dr. Eman Khalaf (res assoc), Angelica Miraples (PhD student)*
  - Applied: to discover, test and breed bacteria that combat Fusarium disease of corn and wheat or reduce the need for nitrogen fertilizer which contributes to climate change. *Michelle Thompson (PhD student), Omar Hewedy (PhD student), Jake Gregory (MSc student), Erik Glemser (tech), Julianna Tindall (undergrad)*
  - International: to empower subsistence farmers to be able to improve and share their own nitrogen-fixing bacteria. *Roshan Pudasaini (PhD student)*
- Methods
  - Culturing, in vitro assays, PACBIO full-length 16S sequencing, WGS, bioinformatics, greenhouse AND field trials
- Vexing question(s)
  - What is the life strategy of plants, given that plants take up copious amounts of microbes from their environment (e.g., soil), in comparison to those that are inherited?
  - Why do beneficial microbes work great indoors, but often fail in the field?

JOEY BERNHARDT

Integrative Biology, CBS

- Research goal
  - Develop a mechanistic understanding of how living systems respond to global change
- Methods
  - Experimental evolution, lab experiments across thermal gradients, flow cytometry, high-throughput phenotyping, microscopy, measurements of metabolic processes, whole genome-resequencing
- Vexing question(s)
  - How do living systems respond to change?
  - How do living systems anticipate change?

REBECCA SHAPIRO

Molecular & Cellular Biology, CBS

- Research goal
  - Understanding genetic mechanisms of fungal-host interactions
  - Identifying microbial inhibitors of fungal virulence
- Methods
  - Fungal genetics (CRISPR)
  - Functional genomic screening
- Vexing question(s)
  - Growing fungi in complex microbial communities
  - Media/conditions for fungal/bacterial growth
  - Accurate identification of fungi in microbiomes

DIEGO GOMEZ NIETO

Clinical Studies, OVC

- Research goal
  - Identify bacterial communities associated with health
  - Understand how dysbiosis can predispose to disease
  - Manipulate the GI microbiota to ameliorate the disease severity, speed recovery and minimize the use of antibiotics
- Methods
  - 16s RNA gene, culturomics, WGS
- Vexing question(s)
  - Has NGS technology helped to identify a new cause of disease in any species?

AYESHA ALI

Maths & Stats, CEPS

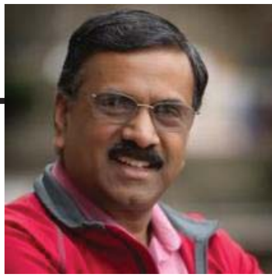
- Research goal
  - Understand how microorganisms interact with each other and their environments
  - Identify important features of the host/host environment that are predictive of their microbial compositions
- Methods
  - Consider interactions within an econometric consumer-preference framework
  - Develop statistical methods for analyzing high-dimensional compositional count data
- Vexing question(s)
  - Which predictors are most relevant for modelling species-host interactions?
  - How do we analyze compositional data across spatial and/or temporal gradients?
  - How much data is needed to draw useful insights from our analysis results?

KARI DUNFIELD

Environmental Sciences, OAC

- Research goal
  - Soil Biodiversity and Health
  - Climate Smart Agriculture
  - Sustainable Food Production
  - Ecosystem Remediation and Environmental Monitoring
- Methods
  - We used field based studies in combination with molecular tools to answer applied research questions about microbially driven ecosystem processes
- Vexing question(s)
  - Exploring strategies to manipulate the microbiome either through introducing novel microbes or microbial consortia or through an ecological engineering approach that enhances the native microbiome by modifying the environment and has the potential to be biological solutions for environmental problems.

JAYASANKAR SUBRAMANIAN



Plant Agriculture, OAC

- Research goal
  - Study the microbiome diversity in *Prunus* spp with emphasis on microbiomes and disease resistance
- Methods
  - Collect samples during various active growth period (May-Oct) and compare the microbiome dynamics using 16s rRNA sequencing
- Vexing question(s)
  - Is there a difference among various *Prunus* spp in microbiome population?
  - Does the microbiome dynamics vary with the phase of growth? E.g. dormant, flowering, active growth, fruiting, senescence stages of the tree