

# INTERNATIONAL PHYTOBIOMES CONFERENCE 2022

I3-I5 SEPTEMBER CABLE CENTER DENVER, CO USA



## International Phytobiomes Conference 2022 Cable Center, Denver, Colorado, USA 13-15 September 2022

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Dear Colleagues,

It is with great pleasure that we welcome you to Denver for the International Phytobiomes Conference 2022.

Over the next three days, agricultural stakeholders, public and private sector agronomists, plant pathologists, geneticists, microbiologists, soil scientists, data scientists, biostatisticians, plant physiologists, nutrient management experts, weather/ climate scientists – to name only some disciplines – will present their research, discuss, and share their expertise to advance knowledge on how phytobiome components interact and affect each other.

Plants grow in association and interaction with complex communities of organisms, environmental conditions, and management practices. The term "Phytobiomes" encompasses all of this complexity. It refers to a plant ("phyto") in a distinct geographical unit ("biome") — a field, grassland, greenhouse, garden, or forest. A phytobiome includes the plant itself, all micro- and macro-organisms living in, on, or around the plant — such as microbes, animals, insects and other plants — and the environment, including soil, air, water, weather, and climate.

Understanding entire systems of phytobiomes is critical to ensuring sustainable global food security in the next decades in the context of population growth, climate change, and the necessity to preserve biodiversity and natural resources. The goal of the Conference is to bring together a broad community of scientists to collectively advance knowledge of phytobiomes aimed at increasing the production of a sufficient supply of safe and nutritious food, feed, and fiber.

The scientific program covers a wide range of topics related to phytobiomes: farm sustainability, interactions between microbial communities and associated plants, soil health, nutrient management and uptake, the use of models and imaging to probe the phytobiome, the development of databases and modeling tools for next generation agriculture and the transformation of phytobiome discoveries into products.

We hope to generate stimulating discussions and initiate collaborations within and across disciplines for the advancement of phytobiomes research.

We would like to thank the Conference partners for their financial support: the International Alliance for Phytobiomes Research, The College of Agricultural Sciences at Colorado State University, the French National Research Institute for Sustainable Development (IRD), NewLeaf Symbiotics, Eversole Associates, Joyn Bio and Syngenta.

We would also like to thank the U.S. National Science Foundation (NSF) for providing travel support for thirteen early career scientists and the U.S. Department of Agriculture's National Institute for Food and Agriculture for their financial support.

We wish you an excellent Conference, stimulating interactions with colleagues, and a great time in Denver.

The Scientific Committee



INTERNATIONAL PHYTOBIOMES CONFERENCE 2022 The International Phytobiomes Conference 2022 is organized by the International Alliance for Phytobiomes Research and Colorado State University.

This is the fourth phytobiomes conference co-organized with partners. The first was held in 2015 in Washington, D.C. (United States), the second - a Keystone Symposia - was held in 2016 in Santa Fe, New Mexico (United States), and the third was held in 2018 in Montpellier (France).

## **Scientific Committee**

- Matthieu Barret, INRAE, FR
- Gwyn Beattie, Iowa State University, US
- Natalie Breakfield, NewLeaf Symbiotics, US
- Trevor Charles, Waterloo Centre for Microbial Research, CA
- Kellye Eversole, Eversole Associates, US
- Gary Felton, Penn State University, US
- Magalie Guilhabert, Bayer Crop Science, US
- Andrew Jones, Colorado State University, US
- Jan Leach, Colorado State University, US
- Ann Lichens-Park, Eversole Associates, US
- Emmanuelle Maguin, INRAE, FR
- Matthew Ryan, CABI, UK
- Angela Sessitch, AIT Austrian Institute of Technology, AT
- Brajesh Singh, Western Sydney University, AU
- Tammy Zimmer, Joyn Bio, US

## Local Organizing Committee

- Amy Charkowski, Colorado State University, US
- Andrew Jones, Colorado State University, US
- Jillian Lang, Colorado State University, US

## **Conference Office – Phytobiomes Alliance**

- Dusti Gallagher (Coordination)
- Isabelle Caugant (Communication)
- Lori Leach (Finance)

## Support

The International Phytobiomes Conference 2022 is supported by the U.S. National Science Foundation under Grant No. 2211046 and by the Agriculture and Food Research Initiative grant no. 2021-67017-35265/project accession no. 1026155 from the USDA National Institute of Food and Agriculture.



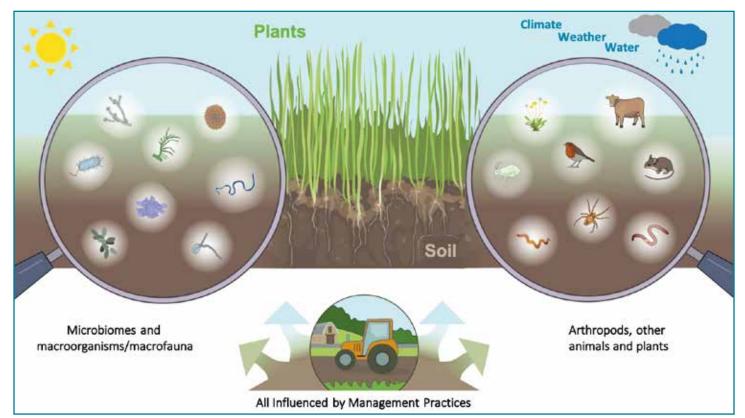


National Institute of Food and Agriculture U.S. DEPARTMENT OF AGRICULTURE Plants grow in association and interaction with complex communities of organisms, environmental conditions, and management practices. The term "Phytobiomes" encompasses all of this complexity.

A phytobiome is a plant ("phyto") in a distinct geographical unit ("biome") - a field, grassland, greenhouse, garden, or forest. A phytobiome includes the plant itself, all micro- and macro-organisms living in, on, or around the plant - such as microbes, animals, insects and other plants - and the environment, including soil, air, water, weather, and climate.

Phytobiomes have an important role in the sustained health and productivity of plants and plant ecosystems.

Because interactions within phytobiomes are dynamic and complex, there is a need to build a foundation of systems-level knowledge of various phytobiomes. This includes an understanding of how the different components interact and influence each other to empower the development of predictive and prescriptive analytics for use in next generation precision agricultural systems.



Establishing a foundation of knowledge on how phytobiome components interact and affect each other will be critical to ensuring sustainable global food security in the next decades in the context of population growth, climate change and necessity to preserve biodiversity and natural resources.



## INTERNATIONAL ALLIANCE FOR PHYTOBIOMES RESEARCH



An international, nonprofit alliance of industry, academic, and governmental partners

## Vision

By 2050, all farmers have the ability to use predictive and prescriptive analytics based on geophysical and biological conditions for determining the best combination of crops, management practices, and inputs for a specific field in a given year

## Mission

Establish a science and technology foundation for sitespecific, phytobiome-based enhancement of sustainable food, feed, and fiber production



Goals

Identify research gaps and help coordinate projects to address those gaps

Establish national, international, and multinational public-private projects and networks

Kellye Eversole Executive Director +1 301-618-9543 eversole@eversoleassociates.com www.phytobiomesalliance.org

@phytobiomes

Phytobiomes Alliance

in internationalphytobiomesalliance



## Venue Address

The Cable Center 2000 Buchtel Boulevard Denver, CO 80210, US



## **Conference Office Contacts**

The registration desk will be staffed for the entire duration of the Conference. Please go to the desk for all your Conference related questions.

Organizers and Student Helpers will be present throughout the conference venue and can be identified by their blue name tags.

Contact email: leach @eversoleassociates.com

## Photographs and Recording

The Conference organizers will take photographs and make videos & audio recordings throughout the conference. By attending the Conference, you hereby grant the organizers the right and permission to use and publish your photo and/or video/audio footage for print and digital communications and marketing.

## **Social Media Policy**

Live-tweeting and sharing of the International Phytobiomes Conference content on social media is welcomed and encouraged as a way to spread information about the Conference. For Twitter, please consider using the conference hashtag **#PhytobiomesConference2022** and tagging **@phytobiomes** 

If a speaker does not wish to have their research shared on social networks, they should make an announcement before and during their presentation.

## Wifi

Available throughout the venue. Network name: TCCGuest

## **Poster Sessions**

Presenters should stand by their posters during the assigned poster sessions on:

- Tuesday 13 September from 17:30 to 18:30
- Wednesday 14 September from 12:30 to 14:00
- Wednesday 14 September from 17:30 to 18:30
- Thursday 15 September from 12:30 to 14:00

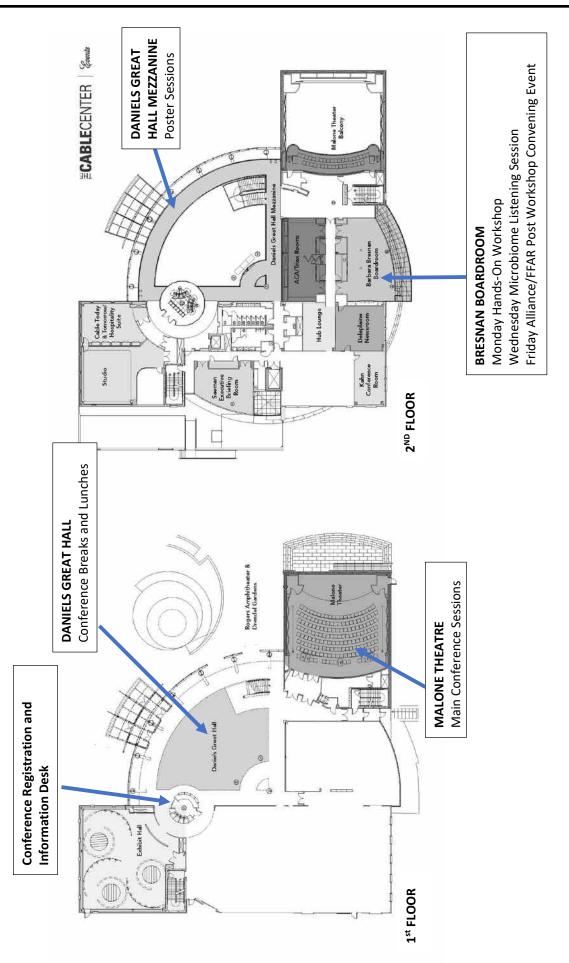
## Best Poster - Public Award

All attendees are encouraged to vote for the "Best Poster" by casting their vote at the registration desk.

## **Online Platform & Networking**

An online platform is available via this QR code to enhance your conference experience. This allows mobile access to the conference program, abstracts, sponsor booths, networking, and more.





## Venue Map

The International Phytobiomes Conference is committed to providing a safe and productive meeting environment that fosters open dialogue and the exchange of scientific ideas, promotes equal opportunities and treatment for all participants, and is free of harassment and discrimination.

This Code of Conduct outlines the Conference organizer's expectations for all meeting attendees, speakers, exhibitors, staff, contractors, and guests at the Conference and social events. All participants in attendance are expected to abide by this Code of Conduct to help ensure a pleasant and safe meeting experience. All participants are expected to treat others with respect, professionalism, and consideration and to uphold standards of scientific integrity and professional ethics.

Conference participants violating these rules may be sanctioned or expelled from the Conference without a refund at the discretion of the Conference organizers.

## Unacceptable Behavior

The following represent types of behaviors that will not be tolerated during the International Phytobiomes Conference and related events:

- Harassment, intimidation, or discrimination in any form.
- Engaging in biased, demeaning, intimidating, coercive, harassing, or hostile conduct or commentary, whether seriously or in jest, based on sex, gender identity or expression, sexual orientation, race, ethnicity, national origin, religion, marital status, veteran status, age, physical appearance, disability, power differential, or other identities.
- Engaging in sexual harassment. Sexual harassment refers to unwelcome sexual advances, requests for sexual favors, and other verbal or physical actions of a sexual nature such as, but not limited to: unnecessary touching; use of sexually degrading words to describe an individual; and display of sexually suggestive objects or images or other materials; and sexually explicit jokes, regardless of the means by which the material is communicated.
- Intentionally talking over or interrupting others.
- Engaging in personal attacks of any kind.
- Commenting on personal appearance.
- Retaliating against persons who file a complaint or assist in an investigation.
- Disrupting the Conference or engaging in harm or threats of harm of any kind.
- Advocating for, or encouraging, any of the above behaviors.

## Reporting

If an individual experiences or witnesses harassment or misconduct, they should immediately report it to the Conference staff identified by their special badges, or contact Lori Leach at leach@eversoleassociates.com or the Conference office at contact@phytobiomesalliance.org. All reports will be treated seriously and confidentially and responded to promptly.

If an individual experience or witnesses harassment or misconduct, it is recommended that, in addition to notifying the contacts mentioned above, they write down the details. They are not expected to discuss the incident with the offending party. Their confidentiality will be maintained to the extent that it does not compromise the rights of others.

All reports will be investigated. We will gather all relevant details and information (verbal or written) from the reporter, the target (if they are not the reporter), and witnesses. Investigations may extend beyond the end of the Conference.

## Enforcement

Conference organizers may take action to redress anything designed to, or with the clear impact of, disrupting the event or making the environment hostile for any participants.

Participants asked to stop any harassing behavior are expected to comply immediately.

If a participant engages in harassing behavior, the Conference organizers retain the right to take any actions to keep the event a welcoming environment for all participants. This includes, but is not limited to, warning the offender or expulsion from the conference with no refund, and barring the person from participating to future Phytobiomes Conferences.

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We expect participants to follow these rules at all event venues and event-related social activities. We think people should follow these rules outside event activities too!

The International Phytobiomes Conference 2022 is made possible by these Sponsors through their generosity and dedication to collaborative science.

**Platinum Sponsor** 



## **Silver Sponsor**



COLLEGE OF AGRICULTURAL SCIENCES COLORADO STATE UNIVERSITY

**Gold Sponsors** 





## **Sponsors**





Gala Dinner Sponsor



## **Media Partners**









## Vanessa Bailey

#### Pacific Northwest National Laboratory, US

Vanessa Bailey is a laboratory fellow at PNNL. Her research focuses on understanding the integrated microbial, chemical, and physical system that comprise soils. To achieve this understanding, she studies how water dynamics within soil pores leads to changes in soil carbon chemistry at pore-, core-, and field scales. This helps resolve the bioavailability of chemically and spatially defined soil carbon to soil microorganisms. Soil microbial communities are a key buffer system against perturbations to

the earth from climate change, pollution, and disturbance. Bailey and colleagues have conducted research showing, for example, that the rate at which microbes are transferring carbon from soil to the atmosphere has increased over a 25-year time period. She also manages one of the Department of Energy's largest coastal research projects, COMPASS, where she is leading efforts to understand the transformations and exchange of carbon and nutrients across the interface between land and sea.



## **Richard Broglie**

#### Pivot Bio, US

Richard Broglie leads Pivot Bio's research and development program, creating a vibrant and innovative R&D culture by championing strong, interdisciplinary connections between scientific specialties. In addition to leading the in-house research teams and managing partnerships with external collaborators, Rich focuses on the acquisition of new technologies, building strong intellectual property portfolios, bringing products to market, working with the leadership team to

develop long-term technology strategies, and mentoring young scientists.

Prior to joining Pivot Bio, Rich spent 30 years in research and global leadership positions at DuPont and DuPont Pioneer and brings extensive experience managing public-private sector partnerships and overseeing global, multi-crop R&D programs. During his tenure, Rich led several discovery programs that resulted in soybean and canola crops with healthier and more stable seed oil compositions. He also developed traits for disease and pest resistance in soybean, corn, wheat, rice, and sugarcane.



## Joseph Cammack

## Farmbox Foods, US

Joseph Cammack is the Executive Vice President of Farmbox Foods. He is experienced in Entrepreneurship, Food Security, and Supply Chain Management. Driven by personal goals to build a sustainable and food safe world, he takes pride in providing mission driven strategies and process improvement practices. As Executive Vice President, his goals include team development, innovation, and eliminating food hunger. Joseph has been honored with the Newel K. Whitney Award for

showing outstanding Character, Diligence and building a better world from Brigham Young University - Idaho.



## Kellye Eversole

#### International Alliance for Phytobiomes Research, US

Kellye Eversole is the Executive Director of the International Alliance for Phytobiomes Research which is pioneering a holistic, systems approach to understanding the complex interactions between the biological and geophysical components of agricultural production systems and support the development of novel, site specific products for enhanced sustainability. She is an expert in agricultural genomics, biotechnology, and information technology research and a leader in the development of international, pre-competitive multidisciplinary, industry-academic research consortia.

## **Invited Speakers**



## **David Glass**

#### D. Glass Associates, US

David Glass is a consultant specializing in industrial/environmental/agricultural biotechnology regulatory affairs, with over 30 years experience with government regulation of the commercial uses of modified microorganisms and plants and other products of biotechnology. David has provided strategic advice to numerous environmental and industrial biotechnology companies regarding compliance with the regulations of EPA, FDA and other agencies. Specifically,

he has advised biofuel and bio-based chemical companies on the applicability of the EPA TSCA regulations to the use of modified microorganisms in fuel or chemical manufacture, he has advised clients on regulatory requirements for several types of FDA-regulated products, including food and feed additives, cosmetics, color additives and dietary supplements (nutraceuticals), and he has assisted in the preparation of submissions for approval of three animal feed ingredients.



## Kirsten Hofmockel

#### Pacific Northwest National Laboratory, US

Kirsten Hofmockel is the President of the Soil Ecology Society, an international organization focused on furthering the science, education and awareness of the importance of soils for human and environmental well-being. She serves as a liaison to the U.S. National Committee for Soil Sciences, which advises the National Academies and represents the interests of the U.S. soil science community in the International Union of Soil Sciences. Hofmockel is a Department of Energy Early

Career Award recipient for research focused on how plants, microbes and soils interact to influence carbon storage. She is a Sr. Scientist at the Pacific Northwest National Laboratory, where she leads the Soil Microbiome Science Focus Area that investigates the basic biology underpinning how soil microbial community members interact to generate beneficial ecosystem functions. Hofmockel holds a joint appointment in the Department of Agronomy at Iowa State University.



## James Hurrell

## Colorado State University, US

James (Jim) W. Hurrell is the Scott Presidential Chair of Environmental Science and Engineering at Colorado State University. He is a former Director of the National Center for Atmospheric Research (NCAR), where he was also a Senior Scientist in the Climate and Global Dynamics Laboratory (CGD). Jim's research has centered on empirical and modeling studies and diagnostic analyses to better understand climate, climate variability, and climate predictability. He currently serves as the Past

President of the Atmospheric Sciences Section of the American Geophysical Union (AGU), and he is a member of Advisory Panel for the Division of Earth and Life Sciences, National Academies of Science, Engineering and Medicine. Jim is a Fellow of the Royal Meteorological Society, the American Meteorological Society, and the AGU.



## Prasanna Kankanala

#### Trace Genomics, US

Prasanna Kankanala is a plant pathologist with two decades of experience in plant-microbe interactions. Her prowess includes molecular interactions between plants and microbes, microbiome analysis, functional studies, whole genome sequencing, high-throughput assay development, and product evaluations. At Trace, Prasanna leads the efforts to innovate on high-throughput systems for development and evaluation of next-generation solutions for agriculture.



## Keith Matthews

#### Wiley Rein LLP, US

Keith Matthews has practiced environmental law focussing on the regulation of chemicals and genetically engineered organisms for over 25 years. He has practiced in the private sector, and for over 13 years was a staff attorney and Assistant General Counsel in the Office of General Counsel at the U.S. Environmental Protection Agency. He then served for four years as the Director of the Biopesticides and Pollution Prevention Division (BPPD) in EPA's Office of Pesticide Programs (OPP).

Now, as Of Counsel with Wiley Rein LLP, his practice focusses on the regulation of chemical products, including biotechnology products regulated by EPA and the U.S. Department of Agriculture. Keith counsels and advises his clients using his breadth of knowledge on a variety of statutes, including the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Federal Food, Drug, and Cosmetic Act (FFDCA), the Toxic Substances Control Act (TSCA), the Endangered Species Act (ESA), and the Plant Protection Act.



## Jennifer Pett-Ridge

#### Lawrence Livermore National Laboratory, US

Jennifer Pett-Ridge is a senior staff scientist and group leader at LLNL who uses the tools of systems biology and biogeochemistry to link, identity, and function in environmental microbial communities and pioneered the use of NanoSIMS isotopic imaging in the fields of microbial biology and soil biogeochemistry. As lead scientist of the LLNL Genomic Science Biofuels Scientific Focus Area (SFA) (2009–2018) and more recently the LLNL Soil Microbiome SFA, she leads multi-disciplinary teams

that integrate biogeochemistry, stable isotope probing, NanoSIMS imaging, molecular microbial ecology, and computational modeling to understand biotic interactions and energy flow in microbial communities critical to soil nutrient cycling and sustainable biofuel production. Pett-Ridge is currently leading a county-level assessment of options for carbon dioxide removal in the USA. She is the group lead for the Environmental Isotope Systems group at LLNL and manages a large portfolio of DOE, NSF, NASA and foundation support. Pett-Ridge has published over 120 peer-review articles, has received a DOE Early Career award, Secretary of Energy Achievement Award, the Geochemical Society Endowed Biogeochemistry Medal, and the DOE Office of Science Ernest Orlando Lawrence Award.



#### **Benjamin Riensche**

#### Blue Diamond Farming Company, US

Benjamin Riensche operates a 16,000-plus acre, sixth generation family farm known as the Blue Diamond Farming Company. Primary operations are in Northeast Iowa where the farm produces corn, soybeans and seed soybeans, and in the Palouse Region of Washington and Idaho where white wheat and chickpeas are grown.

The Riensche Farm seeks to extract value by performing every function involved in their crop

production, from soil fertilization on through production, processing and delivery of every bushel grown. The farm has grown five-fold over the past 28 years of Ben's leadership.

Ben has been farming since 1993. Prior to this, Ben earned an Agricultural Business degree from Iowa State University and an MBA from the University of Chicago. Between school and farming, he worked for Wells Fargo Bank and then for UBS (Switzerland) in their Wall Street, Chicago and Basel, Switzerland offices. He currently serves as the chair of Indigo Agriculture's Strategic Advisory Board, and on the Grower Advisory Panel of Crop Life America.

## **Invited Speakers**



## **Rachel Vannette**

#### University of California, Davis, US

Rachel Vannette is a community ecologist interested in understanding and predicting how microbial communities influence interactions between plants and insects. She studies microbial communities in flowers, on insects, or in soil by combining natural history observations with techniques from chemical ecology, microbial ecology and community ecology. Her work includes applied problems with an immediate application, for example pathogen control or how to landscape to support

pollinators. Other questions may not have an immediate application but are nonetheless grounded in theory and will contribute to basic knowledge and conservation.



## Vittorio Venturi

#### International Centre for Genetic Engineering & Biotechnology, IT

Vittorio Venturi graduated from Edinburgh University, UK in 1988 and received his Ph.D. degree in Microbiology from the University of Utrecht, The Netherlands in 1994. During his Ph.D. research he focused on the regulation of iron-transport processes of beneficial plant associated bacteria which promote plant growth; the monopolization of iron nearby plant roots is an important trait which keeps microbial pathogens away. He then moved as a postdoctoral fellow to the International Centre

for Genetic Engineering & Biotechnology (ICGEB), Trieste, Italy, where he started investigating intercellular signaling among bacteria. He then went on to become Group Leader at ICGEB in 1998 continuing his studies on intercellular signaling. He is now particularly interested in (i) how plant associated bacteria undergo interspecies communication and interkingdom signaling with plants and (ii) plant microbiomes and the development of microbial products for a more sustainable agriculture. Since October 2019 he is also acting as the Scientific Coordinator of ICGEB.



## Alyssa Whitcraft

#### NASA Harvest & University of Maryland, US and GEOGLAM Secretariat, CH

Alyssa Whitcraft is a geographer with broad training in remote sensing, agriculture, and interdisciplinary development studies. She is the co-founder of NASA Harvest, NASA Applied Sciences Program's global food security and agriculture program, for which she co-developed the scientific scope and manages the operations of over 35 projects composed of more than 50 U.S. and international partners from various disciplines and sectors. She is an Associate Research Professor in

the Department of Geographical Sciences at the University of Maryland, and since 2015, she has served as Program Scientist for G20's Group on Earth Observations Global Agricultural Monitoring (GEOGLAM).

Alyssa is an expert in organizational change with respect to integrating new satellite technologies into workflows. She has developed collaborations and partnership models with public and private sector, emphasizing sustainable business models and value to all actors. Having grown up working in her family winery, she understands well the challenges of farm and supply chain resilience in the context of climate change, extreme weather events, and land mismanagement.



#### Kendall Rae Johnson

Kendall Rae Johnson is Georgia's youngest certified farmer. The seven-year-old is the owner of aGROWKulture Urban Farm in Atlanta where she sells food baskets from her own garden, hosts kids marketplace events, and inspires other young people.

#### The Voice of the Future

Her participation in the Phytobiomes Conference is a reminder to all scientists that they are currently working on developing the tools that she will be using twenty years from now. Questions

such as "What does she need?" and "What is the science that we need to focus on?" have to be asked now so that in twenty years farmers like her all over the world, regardless of the size of their farm or the kind of crop they grow, have the tools they need to address the challenges they will be facing, whether it be food security, climate change or agricultural sustainability.

Kendall Rae is very enthusiastic about science and agriculture. She has been playing in the soil since she was three years old. Her love of farming comes from her great grandmother who would say: "Don't throw my collard green stems away, put them back in the dirt." When her parents built a garden bed on their property, Kendall Rae started planting her favorite seeds – cucumbers, peppers, tomatoes and collard greens – and watched them grow. She then would invite her friends over to help harvest her garden, explaining what type of plants she had grown. Today, she services 26 monthly memberships for food baskets from her own garden.

Kendall Rae is currently building an outdoor kids agricultural science learning space. In the coming years, she wants to create an agriculture youth K-12 development program to help all, big or small, grow their own food and learn about food security and urban sustainability. She wants to create a Kids Marketplace where youth entrepreneurs meet the community and learn first-hand how to own and operate a business with help from their parents/guardians, neighborhood business owners, and community supporters.

CUTTING-EDGE RESEARCH World-Class Scientists

Colorado State University is proud to invest in the advancement of agricultural microbiome research and world-class scientists like Jan Leach. By tapping into the potential of the agribiome, we are working to meet global challenges in food safety, food security, wellness and economic prosperity.

We invite you to join us in this effort.





The Early Career Award is awarded to talented early career scientists working on phytobiomes research. The goal of the award is to encourage the participation of students and early career scientists to the International Phytobiomes Conference 2022.

## **Congratulations to the Award Winners**

- Suzanne Fleishman Pennsylvania State University, US
- Albina Khasanova Lawrence Berkeley National Laboratory, US
- Mengying Liu University of Saskatchewan, CA
- Emily Luna Colorado State University, US
- Marco Mechan-Llontop Michigan State University, US
- Bridget O'Banion University of Tennessee, US
- Sarah Richards Pennsylvania State University, US
- Veronica Roman-Reyna Ohio State University, US
- Behnaz Soleimani Julius Kühn-Institut, DE
- Joseph Student University of California, Davis, US
- Abby Sulesky-Grieb Michigan State University, US
- Sherry Sun University of British Columbia, CA
- Tanvi Taparia University of Copenhagen, DK



## **Phytobiomes Journal**

As the official journal for the Phytobiomes Conference 2022, *Phytobiomes Journal* is offering a special publication discount for conference attendees. Attendees can publish up to 12 pages in the journal, at \$150 per page. All articles will undergo peer review before acceptance. To receive this discount, be sure to mention the Phytobiomes Conference in the cover letter.

*Phytobiomes Journal* is a multidisciplinary journal of original research on organisms and communities interacting with plants in any ecosystem.

What makes Phytobiomes Journal unique?

- The first and currently the only journal specializing in phytobiomes research
- Fully open access and highly publicized articles
- Multidisciplinary and international editorial board
- Rapid, high-quality manuscripts and reviews
- Published by APS, a premier organization of the plant and microbial sciences

If you attended the Phytobiomes Conference 2022 and would like to take advantage of this special offer, submit your manuscript to *Phytobiomes Journal* and indicate in Manuscript Central that you attended the conference.



## Tuesday 13 September | 08:00-20:00

| 8:00 - 18:00 | Registration and packet pick-up  | Daniels Great Hall |
|--------------|--|--------------------|
| 9:00 - 9:10  | Welcome, Introductions & Conference Overview<br>Jan Leach, Colorado State University, US   | Malone Theater     |
| 9:10 - 9:55  | Kickoff Plenary – Kellye Eversole, International Phytobiomes Alliance, US<br>Setting the stage for a phytobiomes approach to drive sustainable agricultural production |                    |

## Session 1: Phytobiomes and Farm Sustainability

| Chair: Kellye Eversole, International Phytobiomes Alliance, US |   |  |  |
|--|---|--|--|
| 9:55 - 10:25   | A conversation with <b>Kendall Rae Johnson</b> , Georgia's Youngest Farmer, with <b>Kellye Eversole</b><br>(International Phytobiomes Alliance, US) and <b>LaKisha Odom</b> (Foundation for Food & Agriculture<br>Research – FFAR, US)  |  |  |
| 10:25 - 10:55  | <ul> <li>Producer Roundtable</li> <li>Joseph Cammack, FarmBox Foods, US</li> <li>Ben Riensche, Blue Diamond Farming, US</li> </ul>  |  |  |
| 10:55 - 11:25  | Coffee Break & Networking     Daniels Great Hall  |  |  |
| 11:25 - 11:45  | Talk – <b>Anne Winding, Aarhus University, DK</b><br>Rhizobiome interactions among trophic levels affected by agricultural practices  |  |  |
| 11:45 - 11:55  | <ul> <li>Poster Flash Talks</li> <li>Tanja Kostic, AIT Austrian Institute of Technology, AT<br/>Microbiome-based diagnostic tools for improving primary crop production</li> <li>Jeongyun Choi, Colorado State University, US<br/>Protection of citrus from Huanglongbing by using the antibacterial peptide without threating the inherent<br/>microbiome of leaves</li> </ul> |  |  |
| Session 2: Exploring Interactions Within Phytobiomes           |   |  |  |
| Chair: Jan Leach   | , Colorado State University, US   |  |  |
| 11:55 - 12:40  | Plenary Lecture – Rachel Vannette, University of California, Davis, US<br>Microbial contribution to plant-insect interactions   |  |  |

13:00 - 13:20 Talk – Marco Mechan Llontop, Michigan State University, US Stress-specialized phyllosphere exudates select for distinct microbiome members in sorghum epicuticular wax and aerial root mucilage

Investigating the NHP-dependent SAR pathway in common hexaploid wheat

Talk - Sherry Sun, University of British Columbia, CA



Early Career Award Winner

12:40 - 13:00

| 13:20 - 13:30          | Poster Flash Talks   |                                  |
|------------------------|--|----------------------------------|
|                        | Bridget O'Banion, University of Tennessee, US  |                                  |
|                        | Plant and bacterial inositol exchange influences root colonization outcomes  |                                  |
|                        | Joseph Student, University of California, Davis, US  | <b>6 1 1 1</b>                   |
|                        | Uncovering exometabolites associated with human pathogen survival in the phyllosphe  | re of diverse lettuce            |
|                        | genotypes  |                                  |
| 13:30 - 14:30          | Networking Lunch   | Daniels Great Hall               |
| 14:30 - 15:00          | Talk - Vanessa Bailey, Pacific Northwest National Laboratory, US   |                                  |
|                        | Physical controls on microbial carbon cycling in soils   |                                  |
| 15:00 - 15:20          | Talk – Abby Sulesky-Grieb, Michigan State University, US   | Early Career                     |
|                        | Water limitation and nutrient excess influence the membership and inheritance of the seed  | Award Winner                     |
|                        | microbiome of common bean, Phaseolus vulgaris L.   |                                  |
| 15:20 - 15:40          | Poster Flash Talks   |                                  |
|                        | Anna Garrell, North Carolina State University, US  |                                  |
|                        | Understanding key microbial functions related to maize-microbe interactions using met  | aproteomics                      |
|                        | Lily Cao, Pennylvania State University, US   |                                  |
|                        | Manipulating the soil microbiome and root growth of grapevines with under-vine cover   | crops                            |
|                        | Parul Sharma, Virginia Tech University, US   | •                                |
|                        | Adapting a metagenomic classification tool to reach the resolution required for biosecur   | ity-relevant                     |
|                        | identification of plant-associated bacteria  |                                  |
|                        | Sreejata Bandopadhyay, Michigan State University, US  Impact of drought covarity on accombly of the active rhizesphere microbiome and plant  | matabalita                       |
|                        | Impact of drought severity on assembly of the active rhizosphere microbiome and plant<br>production in common bean and switchgrass: A comparison of direct drought versus pla  |                                  |
|                        |  |                                  |
| 15:40 - 16:10          | Coffee Break & Networking  | Daniels Great Hall               |
| 16:10 - 17:00          | Industry Roundtable: Delivering Grower Solutions   |                                  |
|                        | Fernanda do Amaral, Joyn Bio, US   |                                  |
|                        | Richard Broglie, Pivot Bio, US   |                                  |
|                        | Charles Cowden, Valent BioSciences, US   |                                  |
|                        | Prasanna Kankanala, Trace Genomics, US   |                                  |
| 17:00 - 17:20          | • Frasanna Kankanala, frace Genomics, 05   |                                  |
|                        | Talk – Emily Luna, Colorado State University, US   | Early Corpor                     |
|                        |  | Early Career<br>Award Winner     |
| 17:20 - 17:30          | Talk – Emily Luna, Colorado State University, US   |                                  |
| 17:20 - 17:30          | Talk – <b>Emily Luna, Colorado State University, US</b><br>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bac   |                                  |
| 17:20 - 17:30          | Talk – <b>Emily Luna, Colorado State University, US</b><br>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bac<br>Poster Flash Talks   |                                  |
| 17:20 - 17:30          | Talk - Emily Luna, Colorado State University, US         Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bac         Poster Flash Talks         • Jennifer Shipp, Colorado State University, US  |                                  |
| 17:20 - 17:30          | <ul> <li>Talk - Emily Luna, Colorado State University, US</li> <li>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bac</li> <li>Poster Flash Talks</li> <li>Jennifer Shipp, Colorado State University, US</li> <li>Bacterial blight R gene expression in high temperature environments</li> </ul>  | cteria 🖤 <sup>Award Winner</sup> |
| 17:20 - 17:30          | <ul> <li>Talk - Emily Luna, Colorado State University, US</li> <li>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bac</li> <li>Poster Flash Talks</li> <li>Jennifer Shipp, Colorado State University, US</li> <li>Bacterial blight R gene expression in high temperature environments</li> <li>Kaoutar Bourak, Liege University, BE</li> </ul>  | cteria 🖤 <sup>Award Winner</sup> |
| 17:20 - 17:30<br>17:30 | <ul> <li>Talk - Emily Luna, Colorado State University, US</li> <li>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bac</li> <li>Poster Flash Talks</li> <li>Jennifer Shipp, Colorado State University, US</li> <li>Bacterial blight R gene expression in high temperature environments</li> <li>Kaoutar Bourak, Liege University, BE</li> <li>Impact of phosphorus fertilization on the wheat microbiota as a baseline for microbe-er</li> </ul>   | cteria 🖤 <sup>Award Winner</sup> |
|                        | <ul> <li>Talk - Emily Luna, Colorado State University, US</li> <li>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bace</li> <li>Poster Flash Talks</li> <li>Jennifer Shipp, Colorado State University, US</li> <li>Bacterial blight R gene expression in high temperature environments</li> <li>Kaoutar Bourak, Liege University, BE</li> <li>Impact of phosphorus fertilization on the wheat microbiota as a baseline for microbe-enplant uptake</li> </ul>                                      | cteria 🖤 <sup>Award Winner</sup> |
| 17:30                  | <ul> <li>Talk - Emily Luna, Colorado State University, US</li> <li>Plant defense gene response by Russian wheat aphid honeydew and honeydew-associated bace</li> <li>Poster Flash Talks</li> <li>Jennifer Shipp, Colorado State University, US</li> <li>Bacterial blight R gene expression in high temperature environments</li> <li>Kaoutar Bourak, Liege University, BE</li> <li>Impact of phosphorus fertilization on the wheat microbiota as a baseline for microbe-enplant uptake</li> <li>Wrap-up and Closing Remarks</li> </ul> | eteria 🖤 <sup>Award Winner</sup> |

## Wednesday 14 September | 08:30-21:00

8:30 - 8:40 Welcome & Overview of Day 2 Kellye Eversole, International Phytobiomes Alliance, US Malone Theater

#### Session 3: Soil and Rhizosphere Chair: Ann Lichens-Park, Eversole Associates, US 8:40 - 9:25 Plenary Lecture - Kirsten Hofmockel, Pacific Northwest National Laboratory, US Plant-microbe-mineral interactions within a soil profile 9:25 - 9:45 Talk – Suzanne Fleishman, Pennsylvania State University, US Early Career Award Winner Agroecosystem management modifies the rhizosphere microbiome via soil and host responses: An integrated study of host physiology and root multiomics 9:45 - 10:05 Talk - Mengying Liu, University of Saskatchewan, CA Early Career Impacts of phosphorus fertilizer on the canola (Brassica napus) root and rhizosphere microbiomes Award Winne 10:05 - 10:25 Poster Flash Talks Albina Khasanova, Lawrence Berkeley National Laboratory, US Evaluating plant-microbe interactions: Impacts of metabolic handoffs in the stabilization of synthetic microbial communities • Tanvi Taparia, University of Copenhagen, DK The host-soil-microbiome nexus mediates suppression of bacterial pathogens Kateryna Zhalnina, Lawrence Berkeley National Laboratory, US Linking switchgrass rhizosphere metabolome and microbiome to reveal keystone metabolites under nutrient and moisture limitation Cátia Carreira, Aarhus University, DK The soil RNA virome 10:25 - 11:00 **Coffee Break & Networking Daniels Great Hall** 11:00 - 11:30 Talk – Jennifer Pett-Ridge, Lawrence Livermore National Laboratory, US Life and death in the soil microbiome: How cross-kingdom interactions shape the fate and persistence of soil carbon 11:30 - 11:50 Talk - Melanie Medina, Ohio State University, US Characterization of fungal communities associated with soybean cyst nematode infested fields in Ohio using synthetic long-read amplicon sequencing 11:50 - 12:10 Talk - Nichole Ginnan, University of Kansas, US Recent evolutionary history of the soil microbiome influences plant drought tolerance 12:10 - 14:00 **Networking Lunch & Group Photo Daniels Great Hall**

| 12:10 - 14:00            | Microbiomes Listening Session<br>Ann Lichens-Park, Eversole Associates, US   | Bresnan Board Room                                   |
|--------------------------|--|--|
| 12:30 - 14:00            | Poster Session   | Upstairs Mezzanine                                   |
| Session 4                | : Phytobiomes Engineering  |  |
| Chair: <b>Trevor C</b> l | narles, Waterloo Centre for Microbial Research, CA   |  |
| 14:00 - 14:45            | Plenary Lecture – Vittorio Venturi, International Centre for Genetic Engineeri<br>Communication among microbes in the plant associated microbiome  | ng & Biotechnology, IT                               |
| 14:45 - 15:05            | Talk – <b>Tanvi Taparia, University of Copenhagen, DK</b><br>Soil microbiome regulates trade-offs between productivity and disease pressure for c  | Early Career<br>Award Winner<br>ircular growth media |
| 15:05 - 15:25            | Talk – <b>Soledad Benitez, Ohio State University, US</b><br>System design, surface characteristics and recirculation influence microbial communities in hydroponic leafy<br>green production   |  |
| 15:25 - 15:40            | <ul> <li>Poster Flash Talks</li> <li>Sarah Richards, Pennsylvania State University, US<br/>Farm-scale differentiation of active microbial colonizers</li> <li>Margaret Gaylord, Colorado State University, US<br/>Understanding the interactions of synthetic microbial communities and fungal<br/>control in agricultural crops</li> <li>Stephen Taerum, Connecticut Agricultural Experiment Station, US<br/>Protists facilitate the establishment of symbiotic bacteria in rhizosphere microb</li> </ul> | -  |
| 15:40 - 16:10            | Coffee Break & Networking  | Daniels Great Hall                                   |
| 16:10 - 16:40            | Talk – <b>Richard Broglie, Pivot Bio, US</b><br>Optimizing nitrogen production in root-associated, diazotrophic bacteria   |  |
| 16:40 - 17:00            | Talk – <b>Matthew Ryan, CABI, UK</b><br>Conserving the microbiome – Underpinning phytobiome research   |  |
| 17:00 - 17:20            | Talk – <b>Alejandro del Barrio Duque, Evologic Technologies, AT</b><br>Mycotec – Cutting edge technology to grow filamentous fungi for agriculture   |  |
| 17:20                    | Wrap-up & Closing Remarks  |  |
| 17:30 - 18:30            | Poster Session   | Upstairs Mezzanine                                   |
| 19:00 - 21:00            | Gala Dinner<br>Sponsored by Syngenta<br>Group  | Daniels Great Hall                                   |

## Thursday 15 September | 8:30-16:45

| 8:30 - 8:35 | Welcome & Overview of Day 3                             |  |
|-------------|---|--|
|             | Kellye Eversole, International Phytobiomes Alliance, US |  |

Malone Theater

8:35 - 8:45 Early Career Award Recognitions

## Session 5: Imaging and Modeling of the Phytobiomes

#### Chair: Gwyn Beattie, Iowa State University, US

| 8:45 - 9:30   | Plenary Lecture – <b>James Hurrell, Colorado State University, US</b><br>The untapped potential of earth system models   |
|---------------|--|
| 9:30 - 9:50   | Talk – Veronica Roman-Reyna, Ohio State University, US   |
| 9:50 - 10:10  | Talk – <b>Reinaldo Alcalde, California Institute of Technology, US</b><br>The role of phenazines in regulating phosphate bioavailability in the rhizosphere  |
| 10:10 - 10:20 | <ul> <li>Poster Flash Talks</li> <li>Andie Gonzales, Colorado State University, US<br/>Climate change influence microbial community composition in the rhizosphere of boreal tree species</li> <li>Viet San Le, Deakin University, AU</li> </ul> |

Significance of agroecological practices in enhancing soil health and yields for a sustainable tea production in Northern Vietnam

## 10:20 - 10:50 Coffee Break, Networking

**Daniels Great Hall** 

## Session 6: Regulatory Challenges & Barriers to Solutions

Chair: Tammy Zimmer, Joyn Bio, US

| 10:50 - 11:00 | Welcome and Session Introduction   |
|---------------|--|
| 11:00 - 11:30 | Talk - Keith Matthews, Wiley Rein LLP, US<br>Regulatory challenges & barriers to solutions   |
| 11:30 - 11:55 | Talk - <b>David Glass, D. Glass Associates, US</b><br>Achieving regulatory approval for microbial inoculants or soil additives   |
| 11:55 - 12:25 | Regulatory Roundtable<br>• David Glass, D. Glass Associates, US<br>• Jennifer Lilly, Plant Response, US<br>• Keith Matthews, Wiley Rein LLP, US<br>• Boris Vinatzer, Virginia Tech, US |
| 12:25 - 12:30 | Regulatory Session Wrap-up   |

| 12:30 - 14:00    | Networking Lunch  | Daniels Great Hall              |
|------------------|---|---------------------------------|
| 12:30 - 14:00    | Poster Session  | Upstairs Mezzanine              |
| Session 7:       | Applying Phytobiome-based Solutions   |                                 |
| Chair: Natalie B | reakfield, NewLeaf Symbiotics, US   |                                 |
| 14:00 - 14:45    | Plenary lecture – Alyssa Whitcraft, NASA Harvest & University of Maryla<br>Secretariat, CH<br>A view from above: What satellites can (and cannot yet?) tell us about agricult<br>and climate resilience |                                 |
| 14:45 - 15:05    | Talk – <b>Behnaz Soleimani, Julius Kuehn Institute, DE</b><br>Increased wheat resistance to leaf rust by priming  | Early Career<br>Award Winner    |
| 15:05 - 15:25    | Talk – <b>Angela Sessitsch, AIT Austrian Institute of Technology, AT</b><br>From the ecology and functions of the plant microbiome to its importance in th  | e food system                   |
| 15:25 - 15:55    | Talk – <b>Prasanna Kankanala, Trace Genomics, US</b><br>Decoding soil with whole genome sequencing for scalable & sustainable Ag solu   | ıtions                          |
| 15:55 - 16:15    | Talk – <b>Emerick Larkin, University of Florida, US</b><br>Phosphorous fertilization rates and rhizolocales drive fungal and bacterial rhizo<br>lettuce grown in Pahokee muck                           | community structures in romaine |
| 16:15 - 16:35    | Talk – <b>Veronic Toepfer, Julius Kuehn Institute, DE</b><br>The impact of biostimulants on drought stress tolerance in barley  |                                 |
| 16:35 - 16:45    | Conference Wrap up & Closing Remarks  |                                 |

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| Bandopadhyay, Sreejata    | 102      | Impact of drought severity on assembly of the active rhizosphere microbiome   |
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|                           |          | comparison of direct drought versus plant-mediated effects  |
| Beattie, Gwyn             | 103      | Fine-scale spatial mapping of the soybean root microbiome   |
| Bourak, Kaoutar           | 104      | Impact of phosphorus fertilization on the wheat microbiota as a baseline for<br>microbe-enhanced phosphorus plant uptake              |
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| Carreira, Cátia           | 106      | The soil RNA virome   |
| Carter, Mariama           | 107      | Sticking to thrive: Carbohydrate-binding proteins help Ralstonia solanacearum form biofilms and colonize host plants                  |
| Choi, Jeongyun            | 108      | Protection of citrus from Huanglongbing by using the antibacterial peptide without threating the inherent microbiome of leaves        |
| Compant, Stéphane         | 109      | BIOVEXO, a BBI-JU-H2020 project on biocontrol of Xylella and its vector in<br>olive trees for integrated pest management              |
| Compant, Stéphane         | 110      | Interaction between bacteria and the mycorrhiza-like fungus Serendipita indica<br>to enhance plant resistance against plant pathogens |
| Delp, Drew                | 111      | Root-associated bacterial community changes associated with increasing plant stress   |
| Dörr de Quadros, Patricia | 112      | Pseudomonas strains prevail as putative N-fixers in plant compartments of<br>Ontario crops  |
| Ellis, Martel             | 113      | Influence of nutrient availability and land use on heterosis in maize   |
| Faghihinia, Maede         | 114      | Diversified cropping systems can increase soil and rhizosphere network<br>Complexity  |
| Fautt, Chad               | 115      | SYRINGAE: An online portal for the identification and functional characterization of Pseudomonas syringae isolates                    |
| Garrell, Anna             | 116      | Understanding key microbial functions related to maize-microbe interactions using metaproteomics                                      |
| Gaylord, Margaret         | 117      | Understanding the interactions of synthetic microbial communities and fungal pathogens for disease control in agricultural crops      |
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| Hossain, Md Shakhawat     | 120      | Microbiome analysis revealed diverse and distinct microbial communities from field grown peanut nodules                               |
| Kelley, Brittni           | 121      | Investigating the impacts of drought on root microbiome assembly  |

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# Abstracts



INTERNATIONAL PHYTOBIOMES CONFERENCE 2022



## KN

# Setting the Stage for a Phytobiomes Approach to Drive Sustainable Agricultural Production

## Kellye Eversole

International Alliance for Phytobiomes Research, Eau Claire, WI, USA. Eversole Associates, Arlington, MA, USA

## Abstract

To meet the demands of a global human population expected to exceed 9.6 billion by 2055, crop productivity must improve considerably in the face of a steadily changing climate that presents increased biotic and abiotic stressors. Traditional agricultural sciences have relied mostly on research within individual disciplines and linear, reductionist approaches for crop improvement, production methods, and practices. While advancements have been made in developing and characterizing crop genetics and genomics, the soil characteristics, and beneficial microbe-plant interactions, we have a limited understanding of the biome within which plants are grown and the complex geophysical and biological interactions that determine productivity, sustainability, quality, and resistance to stress. Embracing complexity and deciphering the nonlinear organization and regulation of biological systems will move us towards a systems level, phytobiomes approach. Phytobiome is the holistic concept of a plant growing in a specific biome and all of the interacting geophysical and biological components influencing the productivity or health of a plant. Integrating multi-disciplinary knowledge of all aspects of the phytobiome will enable us to identify meaningful interactions between system actors and provide opportunities to elucidate, quantify, model, predict, modify, and ultimately prescribe the cropping systems, methods, and management practices most suited for a particular farm, pasture, grassland, or forest. This presentation will provide an overview of recent progress in the phytobiomes approach, and propose a path towards more transdisciplinary research programs which will provide growers, farmers, ranchers, and foresters with novel integrative tools and resources aimed at optimizing their productivity, sustainability, and profitability.



## S1T1

## Rhizobiome interactions among trophic levels affected by agricultural practices

<u>Anne Winding</u><sup>1</sup>, Paul Henning Krogh<sup>2</sup>, Mille Anna Lilja<sup>1</sup>, Zivile Buivydaite<sup>1</sup>, Rumakanta Sapkota<sup>1</sup> <sup>1</sup>Aarhus University, Roskilde, Denmark. <sup>2</sup>Aarhus University, Aarhus, Denmark

## Abstract

Interactions among trophic levels in rhizosphere are affected by agricultural practices such as crop, type of fertilizer and tillage, as well as indigenous organisms of the soil and seed. In this study, we hypothesized that agricultural practices such as type of fertilizer and tillage have a dominating effect on the rhizobiome interactions compared to soil and seed. The effects of three fertilizers (pig slurry, nitrogen sulfur chemical fertilizer and urea-ammonium nitrate) were tested in two locations with different soil types – sandy clay in western Denmark and clayey sand in eastern Denmark (approx. 200 km apart) in the growth seasons of 2020 and 2021 of spring barley and winter wheat, respectively, in conventional tillage and no-tillage systems. The effects on interactions among soil organisms including bacterial, fungal and invertebrate communities were studied four weeks after fertilizer application and after crop harvest. We used the substrate induced respiration assay MicroResp® and environmental DNA (eDNA) metabarcoding for characterizing the changes in bacterial and fungal communities. Soil microarthropods were studied by a combination of eDNA metabarcoding and taxonomy-based quantification and identification, while earthworms were assessed by identification and biomass. The results from both growth seasons showed differences in the soil microbial and microarthropod communities between the two agricultural sites and type of fertilizer, which impact the interactions. Conventional tillage versus no-tillage had significant effects on the microbiomes as well as on earthworms. Generally, soil type and common agricultural practices significantly influence the rhizobiome interactions among microorganisms, microarthropods and earthworms.



## S2P1

## **Microbial Contribution to Plant-Insect Interactions**

#### Rachel Vannette

UC Davis, Davis, CA, USA

## Abstract

Plant-associated microbial communities not only affect plant phenotype and fitness but also can alter ecological interactions with other organisms. Microbial communities can directly affect plant physiology and growth and can metabolize plant compounds and produce novel compounds that are detectable to insects. In turn, microbial communities associated with plants can affect insect host plant selection, foraging behavior, and metabolism of plant tissues, with consequences for plant performance and fitness. In this talk, I will discuss a few of the ways in which microbial effects on plant phenotype can cascade to influence plant-herbivore and plant-pollinator interactions. Examples will include how rhizosphere microbial communities affect plant defense against herbivores, and how microbial growth in flowers affect pollinator behavior and plant reproduction. I conclude this talk by highlighting areas for future study.



## Investigating the NHP-dependent SAR pathway in common hexaploid wheat

Sherry Sun<sup>1,2</sup>, Yuelin Zhang<sup>1</sup>, Cornelia Herrfurth<sup>3</sup>, Ivo Feussner<sup>3</sup>, Guus Bakkeren<sup>2</sup>

<sup>1</sup>The University of British Columbia, Department of Botany, 6270 University Blvd, V6T 1Z4, Vancouver, BC, Canada. <sup>2</sup>Agriculture and Agri-Food Canada, Summerland Research & Development Centre, V0H 1Z0, Summerland, BC, Canada. <sup>3</sup>University of Göttingen, Albrecht-von-Haller-Institute and Göttingen Center for Molecular Biosciences (GZMB), Department of Plant Biochemistry and Service Unit for Metabolomics and Lipidomics, Göttingen, Lower Saxony, Germany

## Abstract

Plant systemic acquired resistance (SAR) is a phenomenon whereby the recognition of a local microbial invasion in aerial tissue confers a 'whole-plant' immune response against a wide range of pathogens. For the model plant Arabidopsis thaliana and various other angiosperms, the key metabolites Pipecolic acid (Pip) and N-hydroxylated pipecolic acid (NHP), synthesized by the reductase SAR-Deficient 4 (AtSARD4) and Flavin-monooxygenase 1 (AtFMO1), respectively, are crucial for proper SAR establishment. However, the extent to which NHP biosynthesis contributes to SAR in common hexaploid wheat (Triticum aestivum) remains unclear. Here, we utilized a combination of protein homology, phylogenetic and transcriptomic analyses to elucidate functional orthologs of AtSARD4 and AtFMO1 in wheat. 48 TaFMO1-like and three TaSARD4 candidates were identified, from which representatives were selected for further functional characterization. All three TaSARD4-expressing transgenic  $\Delta$ sard4 Arabidopsis lines generated displayed dwarfism characteristic of autoimmunity, with significant reductions in rosette size (>45%) compared to the Atsard4 deletion mutant. Two TaFMO1-expressing transgenic  $\Delta$ fmo1 Arabidopsis lines revealed a partial recovery in SAR when infected by the oomycete Hyaloperonospora arabidopsidis, indicating possible functional complementation. Furthermore, supplementing 10mM NHP to a local wheat leaf significantly and systemically reduced by 2-fold the proliferation of the biotrophic wheat fungal pathogen, Puccinia triticina (Pt); quantification of basal and Pt-induced NHP and Pip levels in wheat by HPLC-MS is currently underway. Altogether, our results suggest that the NHP biosynthetic pathway for pathogen defense is conserved in wheat and illustrate the power of translating knowledge from model to important non-model crop systems.



# Stress-specialized phyllosphere exudates select for distinct microbiome members in sorghum epicuticular wax and aerial root mucilage

Marco Mechan-Llontop<sup>1,2</sup>, John Mullet<sup>3,2</sup>, Ashley Shade<sup>1,2</sup>

<sup>1</sup>Michigan State University, East Lansing, MI, USA. <sup>2</sup>Great Lakes Bioenergy Research Center, East Lansing, MI, USA. <sup>3</sup>Texas A&M University, College Station, TX, USA

## Abstract

Bioenergy sorghum produces several exudates on its external aerial surfaces to adapt to abiotic stress. Sorghum accumulates elevated levels of epicuticular wax on its stems and leaves to prevent water loss. Also, sorghum produces sugar-rich mucilage on its aerial roots, likely to facilitate nutrient acquisition. We hypothesized that these phyllospheric exudates selectively enrich for specific microbiome members that support host resilience. Here, we assessed the microbiome associated with the epicuticular wax from sorghum plants under non-limiting and limiting water conditions, and the aerial root mucilage from N-fertilized and non-fertilized plants. Our study included two different developmental points over two growing seasons and two field sites in Michigan and Texas. In parallel, we isolated and characterized hundreds of bacterial isolates from wax and mucilage.

We found that the dominant microbial phyla in the wax were Proteobacteria and Firmicutes, and Sphingomonadaceae and Rhizobiaceae families were the major taxa regardless of water availability to plants. The dominant bacterial phyla in the mucilage were Proteobacteria, Bacteroidetes, and Firmicutes. The mucilage-associated microbiome contained described diazotroph bacterial species and exhibited changes over time. We characterized 200 bacterial isolates with putative plant-beneficial traits. By sequencing the genome of several isolates, we observed that the wax isolates harbors genes associated with stress tolerance, and the mucilage isolates are enriched in plant-growth promotion genes. We also found that the Ascomycota and Basidiomycota phyla dominate the mucilage fungal community.

This work integrates data from both cultivation-independent and -dependent approaches to gain deeper insights into phyllosphere dynamics, functions, and host-beneficial phenotypes.



## Physical controls on microbial carbon cycling in soils

## Vanessa Bailey

Pacific Northwest National Laboratory, Richland, Washington, USA

## Abstract

Soil structure, soil water, and soil microbiology interlink to regulate the soil carbon cycle; carbon cycle feedbacks to the Earth system result from the destabilization of soil carbon. Destabilization includes processes that occur along a spectrum through which carbon shifts from a "protected" state to an "available" state where it can be mineralized by microbes to gaseous or soluble forms that are then lost from the soil. The physical structure of soil – aggregates and pores – partially govern these shifts. For example, soil pores comprise the habitat for soil microbes, the flow paths for resource transport in the aqueous phase, and pore water is the reagent within which biogeochemical transformations occur. We combine advanced techniques for molecular characterization of soil carbon with tomography and sequencing to reveal where in the soil matrix carbon persists, and in what forms. We have found little evidence for chemical recalcitrance as a carbon protection mechanism. We have imposed extreme water cycles, from drought through flood, and found that moisture history is a strong control on the forms of carbon in soil, where they are located, and how they contribute to CO2 emitted through heterotrophic respiration. Yet, we find these patterns are expressed differently in different soils and we hypothesize that water and soil structure may explain some of these differences. By considering different physical, chemical, and biological controls as processes that contribute to soil C destabilization, we can inform more accurate and robust predictions of soil C cycling in a changing environment.



# Water Limitation and Nutrient Excess Influence the Membership and Inheritance of the Seed Microbiome of Common Bean, *Phaseolus vulgaris* L.

<u>Abby Sulesky-Grieb</u><sup>1</sup>, A. Fina Bintarti<sup>1</sup>, Marie Simonin<sup>2</sup>, Matthieu Barret<sup>2</sup>, Ashley Shade<sup>1</sup> <sup>1</sup>Michigan State University, East Lansing, MI, USA. <sup>2</sup>INRAE, Angers, Pays de la Loire, France

## Abstract

Plant microbiome interactions play an important role in plant health, including benefits such as water and nutrient assimilation and plant growth promotion. One particularly interesting compartment of the plant microbiome is the seed endosphere – microorganisms residing inside the seed tissues. These seed microbiome members can have short- and long-term consequences on plant fitness, but they are largely understudied. Our study investigated the microbial taxa inhabiting the seed of common bean, and how these taxa are influenced by environmental factors such as drought and exogenous nutrient addition. We applied water limitation and excess fertilizer to bean plants through a multi-generational plant experiment and conducted 16S rRNA amplicon sequencing to characterize the seed endosphere microbiome. With this design, we determined the impact of the same and opposite consecutive treatments over generations. Using these data, we determined treatment and generational effects on the microbiome, differentially abundant taxa across treatments, and inheritance of taxa for each treatment. Host treatment effects were decreased in gen2 as compared to gen1, suggesting potential influence of the previous generation's exposure. We expect these results to increase understanding of key microbiome members responsive to altered environmental conditions, and their persisting impact on the plant microbiome. Ultimately, this work will provide insights into potential agricultural management techniques to increase plant health and resilience under changing climate conditions.



## Plant defense gene response by Russian wheat aphid honeydew and honeydewassociated bacteria

Emily Luna, Santiago Pinedo, Janet Hardin, Jan Leach Colorado State University, Fort Collins, CO, USA

## Abstract

Plant health outcomes are largely based on the dynamic interactions of multiple organisms in the phytobiome. Our previous work demonstrated that variation in Russian wheat aphid (RWA, *Diuraphis noxia*)-induced chlorosis on wheat is determined, in part, by aphid-associated bacteria. Aphids with high titers of bacteria induced gene expression and accumulation of salicylic acid (SA), a hormone that inhibits insect resistance, in susceptible wheat. High, sustained expression of SA biosynthetic genes was followed by downregulation of jasmonic acid (JA) biosynthetic genes; JA is associated with insect resistance. These trends in plant defense gene modulation were also evident in barley, an alternate host for RWA with considerable genetic resources. We hypothesize that aphid-associated bacteria contribute to aphid virulence by modulating the plants' insect defense mechanisms. Although saliva from aphids contains culturable bacteria, scanning electron microscopy (SEM) studies did not detect bacteria in high numbers in the aphid salivary glands or foreguts. However, the same genera of bacteria were detected in aphid honeydew as in saliva; thus, we are focusing on the role of honeydew in induction of plant defense response pathways in barley.



## S3P1

## Plant-microbe-mineral interactions within a soil profile

Kirsten Hofmockel<sup>1,2</sup>, Katherine Naasko<sup>3</sup>, Sneha Couvillion<sup>1</sup>, Emily Graham<sup>1</sup>, Janet Jansson<sup>1</sup>

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## Abstract

The rhizosphere microbiome is critical to plant performance and soil biogeochemical cycling with important global carbon cycle feedback, including plant growth, stress tolerance, and carbon metabolism. Our research aims to determine how the rhizosphere of perennial plants influences microbial structure and function throughout the soil profile and the implications of plant-microbe-mineral interactions on carbon cycling. We conducted our research in a marginal soil, using a multi-omics approach to determine the structure and function of the rhizosphere microbiome, including relationships with abiotic soil properties. Plant inputs influenced the soil microbiome composition and significantly increased the relative abundance of multiple taxa. Although rhizosphere effects on community members were more pronounced than soil depth, we found significant shifts in metabolic signatures throughout the soil profile. On average, the surface soil had significantly higher levels of trehalose and several sugar alcohols known to be produced in response to water, heat, and salt stress. The surface soil also had significantly higher levels of linoleic acid, which was negatively correlated to calcium concentrations and positively correlated to concentrations of soil organic matter and metal micronutrients. In contrast, the deep calcareous soil horizon contained significantly higher levels of organic acids. Soil calcium concentrations were positively correlated with benzoic acid and lactic acid concentrations, suggesting enhanced dissolution of inorganic C. Together these results illustrate the importance of plant-microbe-mineral interactions in generating and retaining organic and inorganic forms of C throughout the soil profile and provide important considerations for managing the rhizosphere to support sustainable biomass production.



## S3T1

# Agroecosystem management modifies the rhizosphere microbiome via soil and host responses: an integrated study of host physiology and root multiomics

Suzanne Fleishman<sup>1,2</sup>, Terrence Bell<sup>2</sup>, Dario Cantu<sup>3</sup>, David Eissenstat<sup>4</sup>, Michela Centinari<sup>1</sup>

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## Abstract

The composition of the rhizosphere microbiome is structured by many factors, including the bulk soil microbial pool, microbe-microbe interactions, and the plant host. Our ability to manipulate the rhizosphere environment for agricultural production will be improved with greater understanding of how management practices may modify the rhizosphere, both by altering the soil environment and/or the host plant physiology. To better understand how management practices modify the composition of rhizosphere bacteria and fungi, we implemented an experiment within a long-term study in a vineyard (Vitis spp.) located in Pennsylvania, USA in 2020. Two management practices are under study at the site: 1) rootstock grafting (101-14 mgt. and Riparia) and 2) increased plant diversity with a grass groundcover (Festuca rubra). Roots and soils up to 1 meter were accessed through root boxes and categorial sources of data (root traits, soil properties, transcriptomics, metabolomics, and microbiome) were analyzed both sequentially and integrated methods. PERMANOVAs on Bray-Curtis similarity indicate that rootstock management practices influence rhizosphere bacterial composition (p<0.001), primarily by host effects. In contrast, groundcover management practices altered soil properties, shifted root growth and increased bulk soil microbial diversity (up to 13%); these shifts may have had stronger consequences for the composition of rhizosphere fungi (p=0.054) than bacteria. Preliminary integrated analyses indicate that in this study rhizosphere microbiota were more strongly structured by bulk soil microbes, root gene expression, and new root growth than soil properties. This study demonstrates that integrating physiology and multiomics can clarify how management practices alter rhizosphere microbiome composition.



## S3T2

# Impacts of phosphorus fertilizer on the canola (Brassica napus) root and rhizosphere microbiomes

#### Mengying Liu<sup>1,2</sup>, Patrick Mooleki<sup>3</sup>, Dave Schneider<sup>4</sup>, Leon Kochian<sup>2,1</sup>, Bobbi Helgason<sup>5</sup>

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## Abstract

As an important source of food-grade and industrial oil, canola (B. napus) plants have high phosphorus (P) demand, but the small seeds are susceptible to P fertilizer toxicity. Optimizing P fertilizer management for canola could be aided by a better understanding of microbial community dynamics under different fertilizer application strategies. We conducted a 2-year field experiment at Scott, SK, Canada, supplying seed-placed P fertilizer at three rates (0, 35 and 65 lb P2O5 ac1) with either 1- or 4-inch openers. Bulk soil, rhizosphere soil and roots were collected at vegetative and peak-flowering stages, with bacterial and fungal microbiomes characterized by amplicon-based DNA profiling.

High-rate P fertilizer only minimally contributed to better yield and biomass, however, plant density was largely reduced at early growth stage, suggesting early season P toxicity. Phosphorus had little effect on alpha-diversity of all samples, while root microbial community structures were impacted by year-to-year variation and plant growth stage, together explaining 23% of the variance in bacterial and 16% in fungal community structures, with a minor impact from P fertilizer. Members of the Gammaproteobacteria and Dothideomycetes classes were most P-responsive bacteria and fungi. Redundancy analysis revealed that soil total P was strongly correlated with root bacterial community structure, and plant biomass was highly correlated with the root fungal community structure at peak-flowering. Under our field conditions, the impacts of P fertilizer on canola root microbiomes were realized through subtle but significant changes in specific P-responsive taxa with minimal changes to the overall bacterial and fungal communities.



### S3T3

# Life and Death in the Soil Microbiome: How Cross-Kingdom Interactions Shape the Fate and Persistence of Soil Carbon

#### Jennifer Pett-Ridge

Lawrence Livermore National Lab, Livermore, CA, United States Minor Outlying Islands

#### Abstract

Soil surrounding plant roots, the 'rhizosphere', is a nexus of biological activity. Stimulated by exudates and root decay, rhizosphere organisms (bacteria, archaea, fungi, fauna, and viruses) interact to move carbon from root tissue to surrounding soil, and ultimately regulate how soil carbon is stabilized. While the concepts of soil food webs are well established, a quantitative and mechanistic understanding of how networks of organisms control dynamics of soil organic matter (SOM) and respond to changing precipitation patterns is only recently emerging. While some bio-interactions may be mutually beneficial, many others are the proximal cause of microbial death and turnover, producing microbial 'necromass' that plays a critical role in the persistence of soil organic matter (SOM). Several factors mediate microbial population dynamics, includ-ing top-down pressure from phage and soil microfauna, and environmental shifts in moisture or resource availability.

I will present evidence from studies where cross-kingdom responses to environmental drivers have follow-on effects for soil carbon—including shifts in resource availability around roots, fungal-bacterial interactions, and microbial community successional shifts during a post-drought wet-up. In all of these systems, stable isotope probing (SIP) helps us assess the active microbial and viral community and quantitatively track plant-derived carbon. These studies suggest that cross-kingdom interactions, involving bacteria, fungi, archaea, protists, microfauna and viruses, shape carbon availability and loss pathways and are differentially influenced by both soil habitat (rhizosphere, detritusphere, bulk soil) and natural fluctuations in the physicochemical environment.



### S3T4

# Characterization of fungal communities associated with soybean cyst nematode infested fields in Ohio using synthetic long-read amplicon sequencing

<u>Melanie Medina López</u><sup>1</sup>, Maria Soledad Benitez Ponce<sup>1</sup>, Horacio López-Nicora<sup>2</sup>, Timothy Ralston<sup>2</sup> <sup>1</sup>The Ohio State University, Wooster, OH, USA. <sup>2</sup>The Ohio State University, Columbus, OH, USA

#### Abstract

Heterodera glycines, the soybean cyst nematode (SCN), is the most economically important pathogen of soybean causing yield losses of over \$1.5 billion in the US alone. Ohio is one of the biggest producers of soybean in the US and SCN has been detected in 82% of its counties. Although the importance of this pathogen is clear, the control measures available are limited to cultural practices and the use of resistant cultivars. Alternative methods, like the use of biological controls, have been studied and fungal groups have shown promising results in addressing SCN infestation. However, the soil microbial communities of soybean fields infested with SCN in the state of Ohio have not been characterized to identify potential biocontrol agents. In this study, we analyzed 170 soil samples with different levels of SCN infestation from 0 to 15,800 SCN eggs per cm3 of soil from 26 counties in Ohio collected in 2019 and 2021. Fungal barcoding ribosomal genes were sequenced using synthetic long-read technology and analyzed to identify the fungal communities present in these soil samples. From our analysis, we found that SCN infestation did not significantly affect the diversity or richness of fungi in the soil. We also learned that SCN infestation was not a primary driver of fungal community composition. While large-scale effects in fungal communities were not seen at different levels of SCN infestation further analysis could uncover fine-scale differences that might point to fungal groups with potential biocontrol activity in the state of Ohio.



# S3T5

# Recent Evolutionary History of the Soil Microbiome Influences Plant Drought Tolerance

Nichole Ginnan<sup>1,2</sup>, Valeria Custodio<sup>3</sup>, Gabriel Castrillo<sup>3</sup>, Maggie Wagner<sup>1,2</sup>

<sup>1</sup>University of Kansas, Lawrence, KS, USA. <sup>2</sup>Kansas Biological Survey and Center for Ecological Research, Lawrence, KS, USA. <sup>3</sup>University of Nottingham, Sutton Bonington, United Kingdom

#### Abstract

Soil microbes can evolve independently in free-living environments or closely intertwined with a plant host. It is unclear whether, or how, a host affects the evolution of microbiomes, and vice versa. We explore this in the context of plant/microbial adaptation to a model stress: water limitation. We performed a mesocosm experiment with six pristine prairie soils collected from across a steep precipitation gradient. Under simulated drought conditions, soil communities from historically dry sites increased root/shoot mass of *Tripsacum dactyloides* (gamagrass), a native prairie grass and relative of maize, as compared to soil communities from wet sites. This suggests that soil microbes are adapted to local precipitation levels and those adaptations impact plant drought tolerance. Next, we experimentally evolved (EE) these soil microbiomes for 5 months in one of four treatments: a factorial combination of +/- water-stress and +/- plant host (gamagrass). EE rhizosphere/soil microbiomes were then inoculated onto maize and gamagrass seedlings to test the functional consequences of microbiome evolutionary history on plant phenotypes under drought. Preliminary results indicate that microbiomes evolved without a host, under drought stress, or both. In contrast, EE microbiomes did not significantly affect maize phenotypes. This suggests that plant-microbiome interactions are more beneficial to plants when they share an evolutionary history with their microbes, but stressful conditions may lead to the breakdown of these relationships. Metagenomic and transcriptomic analyses are underway to unravel the mechanisms driving these phenotypes.



### S4P1

### Communication among microbes in the plant associated microbiome

#### Vittorio Venturi

International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

#### Abstract

Plant associated microbial communities play key roles in biotic and abiotic stress tolerance as well as nutrient acquisition. The rhizosphere (the nearest soil area to the roots) hosts a rich microbial community which provides a series of beneficial outcomes related to plant growth. Plant roots recruit their rhizosphere microbiome from bulk soil and a small number of the microbes then enter the plant colonising the root endosphere. The phylogenetic conservation of rhizosphere microbiomes infers an organized assembly of microbiomes which is directed by mechanisms which are at large unknown. These most likely involve cell-cell interactions amongst microbes, plant-microbe signalling and root exudate effects. Microbial cell-cell communication is a way to dynamically regulate a variety of metabolic and physiological activities in response to the host, environment and microbial neighbors. Plant microbiomes contain a very large number of diverse bacterially produced molecules such as quorum sensing signals, volatiles and secondary metabolites which can play cell-cell signaling roles amongst microbial community, on how the many different bacteria signal each other and what functions are regulated, is very much in its infancy. Understanding the chemical languages that shape the plant microbiome will be very informative on how these communities contribute to plant health and physiology. And will also lead to the development of prebiotic compounds as well as microbial probiotic competence for a more sustainable agriculture of economically important crops.



# Soil microbiome regulates trade-offs between productivity and disease pressure for circular growth media

Tanvi Taparia<sup>1,2,3</sup>, Ed Hendrix<sup>2</sup>, Marc Hendriks<sup>2</sup>, Els Nijhuis<sup>2</sup>, Wietse de Boer<sup>3</sup>, Jan van der Wolf<sup>2</sup>

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#### Abstract

Peat is the predominant growth media in horticulture, despite its large ecological footprint. Modern cropping systems rely heavily on dynamic interactions of the host with microorganisms in the growth media. Yet, the microbiome of the growth media has been largely ignored, in the search for sustainable peat-alternatives. Ecological disease control strategies also suffer from a primitive understanding of the resident microbiome.

In this research, we explored the microbial diversity, activity, composition and interactions of four circular and sustainable alternatives in horticultural growth media, that were used to replace peat. We simultaneously evaluated their physico-chemical properties and agronomical performance. We found peat-based growth media to be a prime determinant of both productivity in the cropping system, as a supplier of beneficial microbes.

Peat-alternatives from agricultural residue streams comprised a unique and competitive microbiome, which produced lower yields. However, it also offered an increased invasion resistance to the cropping system due to niche specialisation within the community, and could defer the establishment of both pathogens and bioinoculants. Sanitary treatment of the peat-alternatives by acidification or steaming significantly increased the yield, while reducing the complexity of the resident microbiome, as well as the presence of endemic pathogens.

In conclusion, circular growing media harbours both harmful and beneficial microbes. The bacterial and fungal microbiome of the growth media governs trade-offs between the productivity and disease pressure in circular horticulture. The sucess of circular and pesticide-free horticulture can benefit greatly from management of the resident microbiome of growth media.



# System design, surface characteristics and recirculation influence microbial communities in hydroponic leafy green production

<u>Soledad Benitez Ponce</u>, Fiama Guevara Guillen, Timothy Frey, Leslie Taylor The Ohio State University, Wooster, OH, USA

#### Abstract

Microbial communities in hydroponic systems are less studied compared to soils. In hydroponics, plant roots are suspended in a defined nutrient solution, and system complexity and microbial diversity are lower than in soil. We have established collaborations with ten commercial leafy green hydroponic producers across Ohio. These facilities range in size (from 23 to 13150 m<sup>2</sup>) and production capacity (up to a million heads per year). In addition, we have access to research Nutrient Film Technique (NFT) and Deep-Water Culture (DWC) units. We are characterizing microbial communities from these systems using culture-based and amplicon-metabarcoding approaches. We observed that the diversity of lettuce-associated microorganisms and microorganisms in nutrient solution depends on system design (e.g., NFT vs DWC). Similarly, sampling location, within the recirculating systems (NFT) influences the diversity of recovered bacteria, with more bacterial genera isolated from nutrient solution at the end of the recirculation system compared to the water source and the nutrient solution input. For biofilms, we observed that the materials in research DWCs' pool liners result in greater biofilm buildup, based on colony forming units, than the NFTs' PVC. However, biofilm samples from NFT surfaces harbored two times more bacterial amplicon sequence variants. Our data represents an initial snapshot of the communities of microorganisms found in leafy green hydroponics. Further analysis will contribute to identifying microbial taxa of relevance for plant growth, and how microbial-microbial and plant-microbial interactions differ between bacteria in nutrient solution compared to those establishing biofilms.



# Optimizing nitrogen production in root-associated, diazotrophic bacteria

#### **Richard Broglie**

Pivot Bio, Berkeley, CA, USA

#### Abstract

While synthetic fertilizers have accelerated crop yields worldwide, most synthetic nitrogen farmers apply is never absorbed by the crops they produce; instead, the nitrogen evaporates into the atmosphere and is washed into nearby waterways that become polluted or become the greenhouse gas nitrous oxide.

Biological nitrogen fixation (BNF) by plant-associated bacteria has the potential to provide a sustainable and efficient source of nitrogen for non-legume crops. However, the abundance of fertilizer and residual nitrogen in agricultural soils has repressed BNF in most rhizosphere microbes. By rewiring the regulatory circuits that control nitrogen fixation and ammonium assimilation, we have developed and commercialized diazotrophic strains that can replace up to 40 pounds of synthetic nitrogen in agricultural corn production.



#### **Conserving the microbiome - Underpinning Phytobiome Research**

Matthew Ryan<sup>1</sup>, Kellye Eversole<sup>2</sup>, Dusti Gallagher<sup>3</sup>

<sup>1</sup>CABI, Egham, Surrey, United Kingdom. <sup>2</sup>International Alliance for Phytobiomes Research, Bethesda, Maryland, USA. <sup>3</sup>United States Culture Collection Network, Bethesda, Maryland, USA

#### Abstract

There is a fundamental need to underpin Phytobiome research through the provision of a supporting infrastructure. The EU Microbiome Support CSA has started to define key requirements, including the development of standards; the need to deposit material and supply cultures, samples and associated data for future research in both academia and industry. Importantly this will also provide a mechanism to protect intellectual property, and help researchers adhere to legislative and regulatory requirements including the Nagoya Protocol of the CBD. Integral to the above is the need to preserve soil and plant samples and their microbiota. Historically, culture collections have used cryopreservation at ultra low temperature and freeze drying protocols to preserve 'axenic' microbes. The microbiome presents a more challenging conundrum – how do we translate the methodology to complex samples that may contain many thousands of different species? The answer lies in our understanding of how microbial cells respond to the stresses encountered during freezing, thawing and recovery and how methods can be optimized to retain physiological and genomic integrity for different taxa and cell types. Using this approach, we can start to predict the components of the microbiome that may retain viability and, importantly, retain their functional potential. In this talk an overview will be provided of how state-of-the-art technologies are applied and adapted for complex microbial samples and synthetic consortia for the UK Crop Microbiome Cryobank and how biobanking, culture collection and data networks can come together to support biobanking for Phytobiome research in academia and industry.



# Mycotec - cutting edge technology to grow filamentous fungi for agriculture

Alejandro del Barrio Duque

Evologic Technologies GmbH, Vienna, Austria

#### Abstract

Filamentous fungi comprise some of the microbes with the highest potential to exert beneficial effects on plant protection and plant nutrition. However, to date humankind can produce only 0.001% of all known filamentous fungi in industrial bioreactors, and consequently only very few species have made it into widespread use in agriculture.

Main challenge to industrial production is their mycelium. While stirred tank reactors (liquid fermentation) shred the mycelium to pieces, solid state fermentation (SSF) is regarded as hardly scalable due to the high risk of contaminations, lack of process control, and laborious product recovery from solid material.

At Evologic Technologies we have developed Mycotec, a novel fermentation process replacing the solids of SSF by a biopolymer, protecting the sensitive mycelium and allowing even conidia to form. Mycotec will enable the production of filamentous fungi at large scale yielding stable, pure and safe fungal products.



### S5P1

### The Untapped Potential of Earth System Models

James Hurrell<sup>1</sup>, Danica Lombardozzi<sup>2</sup>, Gordon Bonan<sup>2</sup> <sup>1</sup>Colorado State University, Fort Collins, CO, USA. <sup>2</sup>NCAR, Boulder, CO, USA

#### Abstract

Climate variability and climate change, including changes in extreme weather, are central to human welfare and prosperity, and the functioning of the biosphere in general. Climate change poses risks to many sectors, including agriculture, water, human health, infrastructure, national security, transportation, energy, forests and ecosystems. With the advent of Earth System Models (ESMs), and especially their inclusion of terrestrial and marine ecosystems and biogeochemical cycles, the climate science community's traditional emphasis on the physical climate has been extended to more multifaceted Earth system prediction, including the biosphere and its resources. ESMs offer an opportunity to move beyond physical descriptors of climate states to societally-relevant quantities such as habitat loss, water availability, disease spread, wildfire risk, air quality, and crop, fishery, and timber yields. In short, ESMs provide the means not just to assess the potential for future global change stresses, but also to determine the outcome of those stresses on the biosphere. Earth system prediction is required to inform sound policy that maintains a healthy biosphere and provides the food, energy, and fresh water needed for a growing global population without further exacerbating climate change. The untapped potential of ESMs is, accordingly, to bring dispersed ecosystem research related to climate processes, impacts, adaptation and mitigation into a common, integrative framework. In this talk, I will summarize the current state and future directions of Earth system modeling and prediction, with a focus on developments of relevance to the phytobiomes community, and discuss opportunities for collaboration.



# S5T1

# Developing metagenomic sequencing as a tool for pathogen surveillance and epidemiology

Veronica Roman-Reyna<sup>1,2</sup>, Taylor Klass<sup>1,2</sup>, Brooke Babler<sup>3,4</sup>, Renee Rioux<sup>3</sup>, Jonathan Jacobs<sup>1,2</sup>

<sup>1</sup>The Ohio State University, Columbus, OH, USA. <sup>2</sup>Infectious Diseases Institute, The Ohio State University, Columbus, OH, USA. <sup>3</sup>University of Wisconsin, Madison, WI, USA. <sup>4</sup>Wisconsin Seed Potato Certification Laboratory, Madison, WI, USA

#### Abstract

Plant disease diagnostics and genetic epidemiology are critical tools for effective pathogen detection, management, and surveillance. Metagenomics is a high-throughput sequencing tool that can document disease dynamics. We hypothesize that metagenomics can track the genetic changes of the pathogen over time, capture the interaction with the host microbiome and explain limitations with current diagnostic tools. To test this hypothesis, we used bacterial soft rot and blackleg of potato, caused by Pectobacterium and Dickeya species. These diseases are challenging for current diagnostic tools because of high pathogen genomic variability, identical symptom development, and co-infections. We conducted a diagnostic metagenomics analysis on several samples collected from symptomatic potato tissues in Wisconsin over 2017–2020. We identified Pectobacterium- and Dickeya-specific sequences from the metagenomic data and assembled partial up to near-complete Pectobacterium and Dickeya genomes from several samples. Through this analysis, we were able to describe the microbial diversity associated with each sample and document virulence-related genes in the microbial population that could serve as a resource for pathogenicity. In symptomatic tissues where traditional molecular techniques failed, metagenomics revealed Dickeya and Pectobacterium species. Metagenomics demonstrates the current limitation of some diagnostic tools to detect the causal agent(s). Overall, these results show that metagenomics is an effective method for potato disease diagnosis with the potential, in the future, to impact seed potato certification. We hope this work can be used as a comprehensive reference for molecular epidemiology and diagnostics by utilizing metagenomics as an incorporated tool in disease management strategies.



### S5T2

### The role of phenazines in regulating phosphate bioavailability in the rhizosphere

<u>Reinaldo Alcalde</u>, Dianne Newman Caltech, Pasadena, CA, USA

#### Abstract

Phosphate (P) is poorly available to plants and soil microorganisms due to its association with minerals. To combat limited P, plants release exudates and/or create beneficial relationships with microorganisms that liberate adsorbed P. The molecular drivers of P solubilization are often considered to consist of (organic) acids and chelators. However, reductive dissolution by redox active metabolites (RAMs) is an overlooked mechanism that may be relevant in soil anoxic microenvironments. Moreover, many RAMs (e.g., phenazines) are up-regulated under P limitation by rhizosphere-associated bacteria, and under controlled batch conditions, can liberate bound P. Here, we investigate whether microbially secreted phenazines can enhance P bioavailability and utilization in the rhizosphere. We describe a microscopy imaging technique that uses light sheet fluorescence microscopy to visualize, in real time, root/microbe interactions at the pore-scale without disrupting the spatial heterogeneity of plant roots (Brachypodium distachyon) and surrounding bacteria (Pseudomonas synxantha 2-79). Using fluorescent transcriptional reporters (i.e., an alkaline phosphatase, a phenazine biosynthesis, and an Fe(II) sensing reporter), we are tracking phenazine regulation during P limitation and testing if this results in the reductive dissolution of iron-oxide-bound-phosphate in a rhizosphere setting. Additionally, we are quantifying the total plant phosphorus concentration via ICP-MS to determine the contribution of phenazine-mediated plant P uptake by contrasting results from a phenazine deficient mutant and a phenazine producing bacterial strain. Our findings will help us assess the ecological relevance of phenazine secretion during P limitation and the potential utility of phenazine producing bacteria as P-solubilizing microorganism in agroecosystems.



# S6T1

# **Regulatory Challenges & Barriers to Solutions**

Keith Matthews

Wiley LLP, Washington, DC, USA

#### Abstract

The regulatory scheme applicable to agricultural inputs in the United States is extremely complex. This is true whether the input is a conventional, microbial, or biochemical pesticide, or if the input is a microbial organism that is intended to enhance the growth characteristics or alter certain phytophysiological responses of a plant. My talk will discuss the various authorities, requirements, and regulatory nuances that can confound the unwary technology developer.



### S6T2

# Achieving Regulatory Approval for Microbial Inoculants or Soil Additives

#### David Glass

D. Glass Associates, Inc., Needham, MA, USA

#### Abstract

Modern genetic technologies have led to increased interest in developing microbial products to improve plant growth or crop yield, including efforts to enhance nitrogen fixation or uptake of other nutrients, or to generally enhance the phytobiomes of important crop species. Those products utilizing genetically modified microorganisms may face regulatory barriers in many countries of the world; and in the U.S., those products not meeting the definition of pesticides might be regulated by the Environmental Protection Agency under its biotechnology regulations under the Toxic Substances Control Act (TSCA). Under these regulations, such products might require agency approval not only for commercial sale, but also prior to any outdoor field testing. Although projects involving the outdoor uses of modified microorganisms will trigger enhanced regulatory scrutiny, it is possible to obtain such approvals through proper planning. This presentation will review the regulations of the U.S. and other countries that would govern development of enhanced microbial inoculants and soil additives, it will discuss applicable precedents of successful approvals, and will present strategies for obtaining needed regulatory clearances for new inoculant or soil additive products.



# S7P1

# A View from Above: What Satellites Can (and Cannot... Yet?) Tell Us About Agricultural Sustainability, Soil Health, and Climate Resilience

#### Alyssa Whitcraft

NASA Harvest, College Park, Maryland, USA. GEOGLAM Secretariat, Geneva, Switzerland

#### Abstract

July 2022 marked the 50 year anniversary of the satellite Earth observing era, whose earliest foundations were in agricultural monitoring. After five decades of slow-but-steady progress, we are experiencing an international, trans-sectoral explosion in the use of satellite data for agricultural assessment. While the number and characteristics of satellite missions proliferates, concurrent advances in cloud computing and machine learning/AI are powering a surge in venture capital and public investment alike in this technology's use for everything from agricultural production forecasting, to on-farm decision support tools, to monitoring, measuring, reporting, and verification of "climate-smart" agricultural management.

As we look ahead to the race to reduce agriculture's climate footprint, and the positing of satellite data as a tool to incentivize and enforce mitigative and adaptive agricultural practice adoption, this is a moment to take stock of from whence we came and what is (and is not yet) possible with top-down view satellite data provide. This talk will explore the evolution of EO for agriculture and open up a dialog on these key questions:

- What can satellites tell us about agricultural sustainability, soil health, and resilience to climate change and extreme weather events?
- How can the view they provide be used to inform policy incentives and on-farm decision making that can promote whole ecosystem health?
- Who gets to decide, and who benefits from the use of this data?



### Increased wheat resistance to leaf rust by priming

Behnaz Soleimai, Andreas Stahl, Andrea Matros, Gwendolin Wehner

Julius Kuehn-Institute (JKI), Federal Research Centre for Cultivated Plants, Quedlinburg, Saxony Anhalt, Germany

#### Abstract

Leaf rust (Puccinia triticina) is the most common rust disease in wheat. Several resistance genes identified in wheat are mostly overcome due to high genetic diversity and adaptability of Puccinia fungi. Priming is proposed to enhance plant's defense against pathogens using beneficial rhizosphere microbes which activates stronger cellular defense responses through induced systemic resistance.

Soil bacteria Ensifer meliloti enhance plant resistance against pathogens by quorum sensing N-acyl homoserine lacton (AHL) causing systemic signaling in plants. This study aims to establish priming in wheat to identify the priming efficiency regarding leaf rust resistance and QTL identification by Genome-wide association study (GWAS).

A diverse set of 200 wheat genotypes was tested for priming efficiency regarding P. triticina in two independent experiments comprising three replications each. Wheat seedlings were treated three times with soil injections of two bacteria suspension (expR+ch overexpressing AHL and transformed E. meliloti carrying lactonase gene attM) at 2, 8, 14 days after planting (dap). Plants were infected with P. triticina at 16 dap. Scores of infected leaf area and infection type were recorded at 12 days after infection.

Results revealed lower relative infection under expR+ch representing a increased resistance by priming. Means for infected leaf area under attM and expR+ch were 12.66% and 11.55%. The ANOVA indicated significant genotype and treatment effects.

GWAS was conducted in GAPIT using mixed linear model to study the association between markers and priming efficiency. Preliminary results reveal one significant (p<0.001) associated marker for priming efficiency on chromosome 1B.



# From the ecology and functions of the plant microbiome to its importance in the food system

<u>Angela Sessitsch</u><sup>1</sup>, Tanja Kostic<sup>1</sup>, Carolina Escobar Rodríguez<sup>1</sup>, Hanna Faist<sup>1</sup>, Friederike Trognitz<sup>1</sup>, Birgit Mitter<sup>1</sup>, Emmanuelle Maguin<sup>2</sup>, Yolanda sanz<sup>3</sup>, Gabriele Berg<sup>4</sup>, Michael Schloter<sup>5</sup>, Martin Wagner<sup>6</sup>, Bettina Schelkle<sup>7</sup>

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#### Abstract

Complex microbiota are found in association with all different plant tissues, from the below-ground organs like roots/rhizosphere to above-ground tissues and reproductive organs. Depending on the tissues, vegetation stage and many other environmental factors, microbial communities are differently composed, driven by different parameters and are also responsible for multiple holobiont functions. In my talk I will address the ecology and functioning of microbiota in different plant tissues, e.g., the ecology and functioning of seed microbiota of *Setaria viridis*, or the potential role of potato root and rhizosphere microbiota in regard to drought stress mitigation and linkage of microbiome and plant phenotypic traits. I will also address application-relevant aspects as well as the role of (plant) microbiomes in the food system. Also, an overview of the EU-funded Coordination and Support Action MICROBIOMESUPPORT targeting food systems microbiomes will be given showing the highlights and achievements of this international collaborative effort towards the use of microbiomes to improve the health and resilience of food systems.



### Decoding Soil with Whole Genome Sequencing for scalable & sustainable Ag solutions

<u>Prasanna Kankanala</u><sup>1</sup>, Maria Mooshammer<sup>1,2</sup>, Tyler Barnum<sup>1</sup>, Matt Goh<sup>1</sup>, Hank Taber<sup>1</sup>, Poornima Parameswaran<sup>1</sup> <sup>1</sup>Trace Genomics, Ames, IA, USA. <sup>2</sup>Pivot Bio, Berkeley, CA, USA

#### Abstract

According to the UN Food and Agriculture Organization (UN-FAO) 2022 report, the land and water resources that farmers rely on are stressed to a "breaking point". Shifting to sustainable agricultural practices will be critical to meet the lofty goal of feeding eleven billion humans by 2050 while minimizing environmental and societal damage. Additionally, 52% of global agricultural soil is already degraded, 34% of which is anthropogenic such as excessive inorganic fertilizer use, livestock grazing and soil compaction. Trace Genomics has developed a comprehensive science-driven Soil Environment Engine - TESS<sup>™</sup> - to provide sustainable solutions to agricultural challenges. This engine combines our extensive database of metagenome sequences and soil chemical and physical properties with our proprietary algorithms and processes to decode DNA sequences into actionable information. Powered by TESS<sup>™</sup>, Trace has developed several scalable solutions for growers by providing them science-backed data for managing fertilizer, chemical and biological applications in the field. TESS<sup>™</sup> also offers objective, analytical solutions to track the impacts of different management practices on soil health over time. We will present case studies that demonstrate the solutions provided by TESS<sup>™</sup> and the benefits gained by our customers.



# Phosphorous Fertilization Rates and Rhizolocales Drive Fungal and Bacterial Rhizocommunity Structures in Romaine Lettuce Grown in Pahokee Muck

<u>Emerick Larkin</u>, Gustavo Kreutz, Melanie Correll, Eban Bean, Germán Sandoya University of Florida, Gainesville, Florida, USA

#### Abstract

Florida is the third leading producer of lettuce in the USA. Most production takes place in Pahokee Muck in the Everglades Agriculture Area (EAA). Phosphorus is an essential macronutrient for production and is typically sourced from mines that have negative environmental impacts. The role of microbes in nutrient acquisition and growth support in crops has become apparent. It is important to investigate how different phosphorous-amendment rates, cultivars, and environmental factors affect microbial rhizocommunities, so that these systems may be better managed. Two romaine cultivars were grown in the EAA across two seasons with conventional, half, and no-phosphorous fertilization, for 16s and ITS rRNA sequencing at harvest. Fungal and bacterial communities were assessed for differences in composition, diversity, and other metrics. SparCC Network models were constructed and analyzed. Alternaria and Curvularia were identified as fungal network drivers in the full fertilization groups but not the other fertilization groups. Field season (trial) and rhizolocale were found to be primary drivers of diversity differences between samples. Across trials and rhizolocales and in order of relative abundance, Proteobacteria, Bacteroidota, Acidobacteria, Chloroflexi, and Firmicutes were the dominant bacterial Phyla, and Ascomycota, Unresolved-Fungi, Basidiomycota, Mortierellomycota, and Rozellomycota were the dominant fungal Phyla. DESEQ2 models identified microbes differentially abundant between HalfP and the other P-rates; and between all treatment groups in the fungal dataset. Although year and plot/location have contributed to the fungal and bacterial community compositions/ structures, significant impacts of fertilization rate on both bacterial and fungal communities in the rhizosphere and rhizoplane were observed.



#### The impact of biostimulants on drought stress tolerance in barley

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#### Abstract

Drought is one of the most important factors for crop losses in agriculture. In addition, the amount of conventional plant protection products decreased dramatically in the last few decades because of their negative ecological consequences. For this reason, biostimulants are gaining more importance because of their environment-friendly property. Biostimulants are products containing natural substances and/or microorganisms leading to increasing abiotic and biotic stress tolerance.

The aim of this study is to investigate the drought stress tolerance as well as the resistance against *Blumeria graminis* infection in barley (*Hordeum vulgare L*) in response to the treatment with different biostimulants. Therefore, ten diverse reacting genotypes were selected to analyze drought stress parameters like water use efficiency and biomass. For the *priming* treatment three biologicals have been selected from a pool of 21 tested substances in a preliminary experiment. Drought stress was induced by 20 % water capacity at the third-leaf stage for six weeks followed by a two weeks re-watering phase to investigate the recovery ability of the drought-stressed plants under greenhouse and field conditions with and without treatment with biostimulants.

Preliminary results show a high repeatability of 0.87 and an increase in biomass under drought stress up to 77% by applying extract of burdock.

Furthermore, the effect of biostimulants on genotype specific performance under drought is tested on a gravimetric based phenotyping facility. The comparison to established reference trials in the field complete the presented investigations with a deeper insight into the effect of the biostimulants on drought response of barley.



# Are We Regulating the Right Ralstonia solanacearum Strains as Select Agents?

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#### Abstract

Potato brown rot caused by the R3bv2 subgroup of *Ralstonia solanacearum* is among the most destructive diseases of this key carbohydrate crop, especially for subsistence farmers in highland tropics around the world. Brown rot is also a potential threat to temperate potato production, so R3bv2 is a highly regulated quarantine pest in North America and Europe. Ideally, only the authentically threatening strains of this pathogen are regulated. However, the R3bv2 subgroup is imperfectly defined and other *R. solanacearum* strains are endemic to the US. Genome sequencing revealed that the ongoing global brown rot outbreak is caused by a near-clonal lineage of R3bv2 that originated in the South American Andes. We hypothesized that this Pandemic Lineage (PL) has spread rapidly out of South America and inflicts large crop losses because it has unique biological traits that make it more successful than other R3bv2 strains from the S. American highlands. We measured one such trait, the ability to wilt potato plants at the relatively low temperature of 22C. We compared cool virulence on 'Russet Norkotah' potato of typical PL strains from Kenya, Guatemala, and Cameroon to that of closely-related R3bv2 strains isolated from the S. American highlands and more distant *R. solanacearum* strains. Although the PL strains aggressively wilted potato at 22C, some non-Select Agent strains were equally virulent. Phylogenetic analyses indicate that cool virulence on potato is a polyphyletic trait in *R. solanacearum*. If cool virulence is not unique to the R3bv2 subgroup, the current Select Agent definition should be revisited.



# Impact of drought severity on assembly of the active rhizosphere microbiome and plant metabolite production in common bean and switchgrass: a comparison of direct drought versus plant-mediated effects

<u>Sreejata Bandopadhyay</u>, Xingxing Li, Alan Bowsher, Robert Last, Ashley Shade Michigan State University, East Lansing, Michigan, USA

#### Abstract

Mitigating the effects of climate-related stress on crops is important for global food security. The microbiome associated with plant roots, henceforth, the rhizobiome, can harbor beneficial microbes that alleviate impacts of stress. However, the factors influencing the recruitment of the rhizobiome during stress are not clear. We conducted a greenhouse experiment aimed to understand rhizobiome member responses to drought across two plant species (switchgrass, common bean), the impact of drought severity on rhizobiome recruitment, and the direct versus plant-mediated consequences of drought on the rhizobiome. We collected field rhizosphere soils from switchgrass and common bean, divided them into planted and unplanted treatments, and exposed them to a gradient of drought severity over time. 16S-V4 rRNA gene amplicon sequencing of the active community revealed differences in the rhizobiome composition of bean and switchgrass. Within each crop, there were differences in rhizobiome community structure and rhizosphere soil metabolite profiles between drought and well-watered, and planted and unplanted treatments. Actinobacteria and Alphaproteobacteria were enriched with drought severity in bean. Furano-diterpenes were enriched in drought-treated switchgrass roots and in drought-treated planted soils. In bean, active communities recruited during drought trended towards increasing dissimilarity to pre-drought condition over time; whereas, in switchgrass, they trended towards a stabilizing effect. Our results suggest that different plants may have different strengths of and potential benefits from their relationship with the microbiome during stress. Of the two plants studied here, common bean may potentially benefit from directed manipulation of microbiome during short-term drought.



# Fine-scale spatial mapping of the soybean root microbiome

Amy Welty, <u>Gwyn Beattie</u> Iowa State University, Ames, IA, USA

#### Abstract

Root microbiome studies generally characterize the collective microbiomes across a root system or of a single root tissue, providing little information on the spatiotemporal heterogeneity of root microbiome assembly. Here, we characterized the assembly of the endosphere and rhizosphere microbiomes of soybean through fine-scale spatial sampling of root tissue from plant emergence to late reproductive stages (VE-R3). Null models indicated clear successional patterns in phylogenetic structure that were linked to both root tissue location and plant growth stage. Hierarchical cluster analysis further indicated a definitive shift in all tissue communities after emergence of the first trifoliate leaf (V1), which corresponded to observed changes in plant nodulation and aboveground biomass, and likely to changes in nutrient demand and uptake. Transitions in assembled communities were pronounced in the root tips, which harbored distinct communities at different stages of plant development. We observed a near complete turnover in the core microbiome of root tips before V1 were dominated by ASVs from two copiotrophic genera, Massilia and Paenibacillus, but these taxa were thereafter displaced by a more complex community. In contrast, the subcrown region developed a core microbiome as early as 4 days post-emergence, and showed smaller, incremental changes in peripheral taxa over time. Collectively, the data illustrate tissue-specific root microbiome restructuring linked to plant developmental processes.



# Impact of phosphorus fertilization on the wheat microbiota as a baseline for microbeenhanced phosphorus plant uptake

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<sup>1</sup>Liege University, Terra Research Center, Integrated and Urban Plant Pathology laboratory, Gembloux Agro-Bio-Tech, Gembloux, Belgium. <sup>2</sup>Mohammed VI Polytechnic University, Benguerir, Morocco

#### Abstract

Given that phosphorus (P) is a limited, nonrenewable resource and the efficiency of P fertilizers does not exceed 25%, fertilization practices applied nowadays require a better efficiency of P fertilizers. SoilPhorLife project relies on an international collaboration and focus on efficient use of fertilizers. A part of this project is dedicated to the interplay between plant associated microbes and P uptake.

Plant associated microbiota can enhance nutrient uptake and increase plant resistance to abiotic stresses including nutrients deficiencies Thus, combining plant-associated microbes with P fertilizers represents a promising strategy for a productive and sustainable agriculture. However, the effect of P formulations on soil microbiota structure remains ambiguous. Hence, deep understanding of this interaction could bring insight into how to better manage microbial communities to promote beneficial plant-microbiome interactions for P uptake.

A greenhouse experiment was carried out on bread wheat using agricultural P deficient soil to assess the effects of P fertilizer application on wheat associated microbiome. Thus, different phosphorus formulations (Polyphosphates and Orthophosphates) were used, to unravel their effect(s) on the dynamic and composition of wheat microbiota in the aim of developing microbiological strategies in terms of P uptake. The global purpose of this study was to quantitatively characterize the bacterial and fungal rhizosphere microbiota composition using High throughput sequencing at different physiological stages.

The overall analysis of alpha and beta diversity showed differences among fertilized and non-fertilized wheat rhizosphere during growth stages. These results underlined a diversity of wheat microbiota that was changed during the growth period.



# Manipulating the soil microbiome and root growth of grapevines with under-vine cover crops

#### Lily Cao<sup>1,2,3</sup>, Suzanne Fleishman<sup>1,2,4</sup>, David Eissenstat<sup>1,4</sup>, Michela Centinari<sup>1,2</sup>, Terrence Bell<sup>1,3</sup>

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#### Abstract

Cover crops have been used with cash crops to enhance plant diversity and beneficial agroecosystem services. However, we have limited understanding on how they affect perennial crops. Unlike in annual systems, cover crops for perennial systems often require co-growth, leading to long-term direct interactions. We understand little about how the resulting belowground interactions influence soil microorganisms, root growth, and resource availability. Our study looked at how between-plant root interactions and nutrient resources affect the soil microbiome and root growth. We conducted a field study of perennial Noiret grapevines and grass cover crops, where grass was transplanted above grapevine roots, with organic nitrogen fertilizer added to half the samples to relieve nutrient competition. We hypothesized that grapevine and grass root interactions would 1) shift the soil microbiome composition, 2) affect grapevine root growth, and 3) have decreased impact on soil microbiome composition and root growth with nutrient amendments. Cover crops shifted rhizo-sphere bacterial composition only in the absence of nitrogen amendment but did not impact rhizosphere fungal composition. Cover crops shifted both bacterial and fungal composition in bulk soil. Cover crops increased the Shannon diversity of fungi in soil without nitrogen amendments. The specific root length of grapevines was lower with cover crops but did not differ due to nitrogen amendment. These results suggest cover crops can impact soil microbiome composition and reduce root growth at varying levels depending on soil nutrient competition. Understanding belowground interactions can allow us to manipulate the soil microbiome and root health to improve agricultural production.



# The soil RNA virome

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### Abstract

Viruses are the most abundant entities in soils with about 1 billion viruses g-1 of soil and suggested to be important in promoting biodiversity and affecting nutrient cycling. Currently, there are few surveys of RNA viral communities, with most soil viral research focusing on DNA viruses, although there is indication that the viral RNA community could be very diverse. In this study we determined the genomic diversity of the RNA viral size fraction in four soil types (beech and pine forest, grassland and agricultural) together with physicochemical variables. Viral abundances were highest in the beech forest and lowest in the agricultural soil. Lenarviricota virus dominated in the forest and grassland soils, in particular the bacteriophage *Emesvirus*, while the phylum Kitrinoviricota (mainly plant hosts) dominated in the agricultural soil. Pisuviricota, a phylum that includes plant and marine microbial hosts, was found in higher proportion in the grassland soil that is occasionally flooded with marine water. This study clearly indicates that different soils harbour very different RNA viral communities, but their impact in plant ecology still needs to be determined.



# Sticking to Thrive: Carbohydrate-binding proteins help *Ralstonia solanacearum* form biofilms and colonize host plants

Mariama Carter<sup>1</sup>, Sofia Weinstein<sup>1</sup>, Jessica Prom<sup>2</sup>, Shabda Gajbhiye<sup>1</sup>, Caitilyn Allen<sup>1</sup>

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#### Abstract

Lectins, carbohydrate-binding proteins, mediate critical host-bacterial interactions including adhesion to host cell surfaces, interbacterial cohesion, and biofilm formation. *Ralstonia solanacearum* (*Rs*) encodes three lectins that are among the most highly expressed genes in rich media and in planta: mannose-binding lectin LecM, fucose-binding lectin LecF, and xylose-binding lectin LecX. Given that polysaccharides are integral components of both plant and bacterial cell surfaces, we hypothesize that the lectins function in adhesion to the host and/or cohesion between *Rs* cells, aiding in biofilm formation and host colonization. In comparison to rhizoplane colonization, one of the first steps in bacterial wilt disease, all three lectins were upregulated during root endosphere and stem colonization. In plant colonization studies with individual lectin mutants, neither *lecF* nor *lecX* were required for root endosphere colonization, but *lecF* did contribute to colonization of the stem. *In vitro* biofilm assays revealed that  $\Delta lecF$  formed less biofilm than wild-type, while  $\Delta lecX$  hyper-produced biofilms on PVC plates. The expression of *lecF* is increased in  $\Delta lecX$  in comparison to the wild-type, offering a possible explanation for the surprising hyper-attachment phenotype observed. Together, our results show that lectins contribute to critical attachment behaviors in *Rs* and hint at a regulatory link between the lectins.



# Protection of Citrus from Huanglongbing by using the antibacterial peptide without threating the inherent microbiome of leaves

#### Jeongyun Choi

Microbiome Network and Department of Agricultural Biology, Fort Collins, Colorado, USA

#### Abstract

Citrus greening (aka Huanglongbing, HLB) caused by the phloem-limited α-proteobacteria 'Candidatus Liberibacter asiaticus (CLas) is the most devastating citrus disease in the world. To date, neither a cure nor an economically viable disease control measure is available to the citrus growers. The impact of HLB on the global citrus industry is quite severe. In particular, the Florida's citrus industry risks losing 80% of its production. Our team has designed antibacterial (called a/b) peptides consisting of a helical and b strand segments. Using detached leaf assays, we first tested the efficacy of one of the a/b peptides (UGK-17) to reduce CLas numbers. The AMP application significantly (P = 0.005) reduced CLas numbers in 2 different citrus varieties at 5 and 25 mmol concentrations. The large-scale application of the a/b peptides will require studies on their off-target effects. To this end, we are studying the effects of a/b peptide application on the structure, network properties, and functions of the plant-associated microbiome using amplicon sequencing. We envision that the novel a/b peptides will provide a way forward to design and apply peptides and proteins for effective, affordable, and safe treatment and prevention of HLB.



# **BIOVEXO**, a **BBI-JU-H2020** project on biocontrol of *Xylella* and its vector in olive trees for integrated pest management.

#### Stéphane Compant

AIT Austrian Institute of Technology, Center for Health and Bioresources, Bioresources Unit, Tulln, Austria

#### Abstract

*Xylella fastidiosa* is increasingly causing diseases on olive trees and various other crops in the Mediterranean region. It wiped out a number of olive groves in Italy and Spain in only a few years. Unfortunately, the climate of the southern European Union is ideal for *Xylella*. Due to its rapid transmission across cultivation areas, *Xylella fastidiosa* is projected to cause yield losses of 35% - 70% in olive harvests and 13% in almond harvests. Currently, there are no products available on the market that have been proven to be effective against *Xylella fastidiosa*, which is spread by xylem-feeding insects – notably the spittlebug *Philaenus spumarius*. In the BBI JU-H2020 project BIOVEXO, 11 partners from 5 different countries and belonging to industries, SMEs, RTOs and universities aim to develop environmentally sustainable and economically viable plant protection solutions that can be deployed as a method of integrated pest management to control *Xylella fastidiosa* and its spittlebug vector. At least, the two best performing solutions will be brought forward, closer to the market, at the end of the project (Technical Readiness Level 7-8). Integrated pest management measures will be applied to the existing, and the newly planted orchards during the large-scale pilot in Apulia (Italy) and Balearic islands (Spain) — the two most dominant Xylella outbreak regions in Europe. In the conference, we will present the overview of the project as well as the objectives and the research plan of BIOVEXO.

For more information about the BIOVEXO project: www.biovexo.eu.



# Interaction between bacteria and the mycorrhiza-like fungus *Serendipita indica* to enhance plant resistance against plant pathogens

Alejandro Del Barrio Duque<sup>1</sup>, Livio Antonielli<sup>2</sup>, Angela Sessitsch<sup>2</sup>, Ole Nybroe<sup>3</sup>, Stéphane Compant<sup>2</sup>

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#### Abstract

*Serendipita indica* (syn. *Piriformospora indica*) is a root colonizing endophytic fungus that holds capabilities to enhance plant growth and to confer resistance to different stresses. This fungus is further known as hosting a bacterial endosymbiont, *Rhizobium radiobacter*, living inside its hyphae. However, there are still a gap of knowledge if other bacteria can also have positive effects on the fungus. We isolated and taxonomically characterized nearly 500 bacterial endophytes from potato and tomato roots and studied the impact of individual bacterial isolates on the in vitro growth of the fungus. We further analyzed how co-inoculations of bacteria and *S. indica* influence plant growth and protection against fungal pathogens. Several isolates of *Mycobacteriaceae*, *Rhizobiaceae*, *Xanthomonadaceae* or other families stimulated *S. indica* growth. Some of these bacteria in combination with *S. indica* can further help to reduce tomato diseases caused by *Fusarium oxysporum* and *Ralstonia solani*. Possible mechanisms behind these interactions were described based on genome and advanced microscopic analyses. Bacteria-fungus seem to cooperate in the process of fungal root colonization and establishment: Interestingly, a strain of *Trinickia* colonizes internally spores of *S. indica* as an endofungal bacterium in *in vitro* co-culture, suggesting that the fungus might acquire formerly unrecognized genera of bacteria. Genome analysis of the positive bacteria revealed many genes potentially involved in fungal and plant growth stimulation, biocontrol and root colonization, highlighting putative mechanisms of plant-fungal-bacterial interactions. Our study represents an important step towards the unraveling of the complex interactions among plants, *S. indica*, endophytic bacteria and fungal pathogens.



# Root-associated Bacterial Community Changes Associated with Increasing Plant Stress

Drew Delp, Amy Welty, Gwyn Beattie Iowa State University, Ames, IA, USA

#### Abstract

Drought poses a serious threat to agriculture worldwide and may be mitigated by the activities of plant-associated microbial communities. Drought is known to enrich for bacteria in the Actinomycetota phylum in the root endosphere, but little is known about the mechanisms driving this enrichment. We hypothesized that this enrichment is due, in part, to drought-induced oxidative stress. To test this, we evaluated shifts in the endosphere microbiome in response to abiotic stresses that, like drought, induce reactive oxygen species accumulation in the root apoplast. We used a soybean split-root system in which one set of roots was exposed to stress, namely salinity or copper, and the other to a non-sterile field soil, thus exposing root microbes only to systemic changes from stress. Through microbiome profiling we demonstrated that phylum-level taxonomic shifts previously associated with drought were recapitulated by salinity, and to a lesser extent copper, consistent with oxidative stress driving microbiome shifts during drought. We identified taxa abundance patterns across five treatments that collectively imposed a stress gradient and found that the absolute abundance of some taxa correlated with increasing plant stress; these included the genera Terrabacter, Pseudarthrobacter, Nocardioides, and Glycomyces in the Actinomycetota phylum. These genera were also differentially abundant in the root endosphere versus the rhizosphere, suggesting selective recruitment or differential survival during abiotic stress exposure. Members of the Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium cluster experienced the greatest decrease in absolute abundance with increasing plant stress. These results provide insight into the taxa most influenced by stress-associated changes in plants.



# *Pseudomonas* strains prevail as putative N-fixers in plant compartments of Ontario crops

<u>Patricia Dörr de Quadros</u>, Mozhgan Sepehri, Brittney Lato, Solmaz Komijani, Jesse Branje, Michael Lynch, Jiujun Cheng, Trevor Charles

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#### Abstract

Nitrogen (N) is considered the most expensive nutrient for crops, and it is required in large amounts for the achievement of high crop productivity. With recognized financial and environmental implications for using synthetic N fertilizers, and in consideration of scarcity in the market due to the current global economic and political conditions, the importance of strengthening the use of microbial fertilizers containing N-fixers is paramount. Free-living N-fixing bacteria such as Azospirillum can replace the use of N fertilizers by up to 40%. Most of the microbial N-fixing products used in Canadian agriculture are imported from other countries. Therefore, we set out to identify and isolate bacterial N-fixer strains in Ontario soils. More specifically, the study involved the isolation of microbes from the rhizosphere, roots, and leaves of canola, wheat, barley, soybean, tomato, potato, and peas using a nitrogen-free semi-solid (NFB) medium. The isolation of N-fixers was performed by successively plating on solid NFB, TSA, and Congo red medium (for detection of Azospirillum). In total, 57 bacterial isolates were purified. The 16S rRNA gene was amplified for identification of each isolate, and complete genome sequences were determined by Illumina MiSeq and or Oxford Nanopore MinION. Surprisingly, 31 of the 57 isolates belonged to the genus *Pseudomonas*. Genomic analysis for detection of *nifH* gene are being run. Although *Azospirillum, Herbaspirillum*, and *Acetobacter* are very common genera in tropical soils, Ontario soils indicate the prevalence of putative N-fixing *Pseudomonas*. This finding will support the future design of beneficial microbial inoculants for Ontario crops.



# Influence of nutrient availability and land use on heterosis in maize

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#### Abstract

Heterosis refers to superior phenotypic traits such as emergence, biomass production, and resistance to stress in hybrids relative to their inbred parents. Heterosis has been suggested to be controlled by genetic mechanisms, including the dominance and overdominance hypothesis, as well as molecular mechanisms. Recently, an ecological mechanism has been identified whereby soil microbes differentially effect the growth of hybrid and inbred maize. While manipulation of the soil microbial community consistently altered heterosis, the effect appeared to be dependent on the microbiome composition, as well as the environment. Here we explore how nutrient availability and land use influences the expression of heterosis in three maize genotypes. To accomplish this, we grew B73, Mo17, and B73xMo17 in an agricultural and prairie soil and fertilized them with a high and low concentration nutrient solution. After four weeks of growth, plant traits such as emergence, height, biomass, chlorophyll content, and stem diameter were measured. Since nutrient availability is important for plant growth and development, we expect that all genotypes will be positively affected by the addition of nutrients. Previous preliminary research has shown that heterosis was greater in this prairie soil than the agricultural soil, therefore we expect more heterosis in the prairie soil under both nutrient treatments than in the agricultural soil. Future experiments should explore how specific groups of microbes, such as diazotrophs, are impacted by varying nutrient availability and thereby impact heterosis.



# Diversified Cropping Systems Can Increase Soil and Rhizosphere Network Complexity

#### Maede Faghihinia

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#### Abstract

Cropping system diversity can provide yield benefits that may result from shifts in the composition of root-associated microbial communities, which either enhance nutrient availability or limit nutrient loss. We used random matrix theory-based network analysis of the total (DNA-based) and potentially metabolically active (RNA-based) bacterial communities in the soil, and rhizosphere in two cropping systems. Soil from a conventional (2-year rotation, inorganic fertilization) and a diversified system (4-year rotation, manure amendments) was used to grow maize in microcosms to a developmental stage when the peak rate of nitrogen uptake begins. While modularity was greater in the bulk soil and rhizosphere of conventional systems the networks were more complex and more interconnected in the diversified system. Covariations were predominantly positive (80-99%) for the DNA-based community profiles but less positive (65-78%) for the RNA-based community, suggesting that there is potentially more competition among bacteria than would be assumed from simply monitoring the 16S rRNA gene. Putative keystone taxa represented various phyla and a large proportion (15-20%) of the keystone taxa in the DNA-based profiles. Examination of network node properties revealed that ammonia oxidizers had more links and significantly greater betweenness and eigenvector centrality in the diversified system. This suggests that bacterial communities are more interconnected in diversified than conventional systems and that ammonia oxidizers may play an important role in shaping the soil and rhizosphere microbiome.



# SYRINGAE: An online portal for the identification and functional characterization of Pseudomonas syringae isolates

Chad Fautt, Estelle Couradeau, Kevin Hockett

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#### Abstract

The *Pseudomonas syringae* species complex (PSSC) contains over 15 named species that, collectively, can cause disease in over 80 known plant species, including almost every food crop. As a threat to global food security, it is important to be able to rapidly detect epidemic strains and study the emergence of new pathogenic lineages. To that end, using publicly available genomes, we have assessed the *in-silico* amplification rate and classification resolution of 16 commonly used PCR primer sets. We find that only nine primer sets successfully amplified the desired sequences in more than 50% of genomes, but among these nine primer sets, mean classification resolution was similar, at 97-98% average nucleotide identity. Further, we find that classification resolution is sufficient to predict the content of an unknown isolate's type III effector protein repertoire, a central determinant of virulence, with 80-94% accuracy. Finally, we introduce SYRINGAE.org, featuring a web-based portal that allows for rapid identification and functional characterization of unknown PSSC isolates, as well a dashboard for exploring a 2,161-genome annotated phylogeny of PSSC. SYRINGAE fulfills a need within the plant pathology research community by bringing together data and knowledge generated over the last several decades, improving accessibility and exploration of said data, and leveraging it for accurate, reproducible identification of unknown PSSC isolates using marker genes.



# Understanding key microbial functions related to maize-microbe interactions using metaproteomics

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#### Abstract

Plant root-associated microbes have been shown to provide a number of benefits to their hosts including increased drought tolerance, nutrient availability, and pathogen resistance. While the mechanisms behind these beneficial interactions have been and continue to be investigated, there remains a gap in our understanding of the physiological and metabolic adaptations of these root-associated microbes to the root environment. In order to address this, we grew the members of a synthetic community from the maize root *in vitro* and *in vivo* and used metaproteomics to identify and quantify differentially abundant proteins related to key microbial functions in root-microbe interactions. We identified a number of proteins that were significantly upregulated during *in vivo* growth on corn roots when compared to *in vitro* growth, including proteins in-volved in xylan and fatty acid metabolism, chemotaxis, motility, and secretion systems, as well as enzymes that may indicate iron and vitamin deficiencies in the corn root. These results provide an insight into the physiological characteristics that are important for microbial persistence and survival in the corn root, and may additionally indicate environmental conditions within and around the root. This could have downstream implications for understanding the mechanisms underlying microbial colonization of plant roots, determining the impacts environmental stresses have on plant-microbe interactions, and improving persistence of microbial bioinoculants in the field.



# Understanding the interactions of synthetic microbial communities and fungal pathogens for disease control in agricultural crops

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#### Abstract

Fungal soil pathogens can have devastating effects on crops and be challenging to manage in the field. It is becoming increasingly clear that potential applications for plant protection depend on the understanding and manipulating multispecies interactions that are critical for microbiota-mediated fungal disease resistance. Recent research suggests that defined synthetic microbial communities (SynComs) could confer more efficient plant protection than individual strains. Our overarching goal is to design and formulate SynComs consisting of a consortium of bacterial isolates possessing multiple beneficial traits that can antagonize various fungal pathogens and prime the plants for disease resistance. Using dual culture assays, we have screened 8 bacterial isolates for their antagonistic activities against *Rhizoctonia solani*, causing Rhizoctonia root rot, and *Fusarium oxysporum*, causing Fusarium yellows. These bacteria belonged to various species of *Bacillus*, *Pseudomonas* and *Pantoea*. We are now using microscopic, genomics, and transcriptomics approaches to understand the interaction mechanisms between selected bacteria and fungal pathogens. A detailed understanding of the interactions between beneficial bacteria and pathogenic fungi will allow us to construct effective biocontrol SynComs leading to sustainable management of important crop diseases.



## Comparing Microbiomes from Herbarium and Silica-dried Leaves of Grindelia ciliata

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#### Abstract

An herbarium is a collection of pressed, dried plant specimens and has data used for many long-term scientific studies. Researchers would like to sample the microbiome from herbarium specimens to evaluate how microbiomes have changed over time and across the plants' ranges, as well as examining the impact of climate change on plant microbiota. However, the microbiome of herbarium specimens can change during preparation, mounting, and storage, so it is not clear how representative the microbiomes of herbarium specimens are of the microbes that were on the plants in life. We are examining the effects of herbarium preservation on the leaf microbiome of Grindelia ciliata (gumweed, Asteraceae). It is a native annual or biennial herb commonly found across central United States in grasslands, prairies, and roadsides. We are analyzing the microbiomes of leaves taken from herbarium specimens and leaves that were dried with silica gel immediately after collecting. We are using silica-dried leaves as a proxy for freshly collected leaves. In total, we are examining five individuals each from nine populations collected from throughout range of the species. For each individual, we are sequencing both silica-dried leaves and leaves from herbarium samples. We will use amplicon sequencing to examine the V4 region of the 16S rRNA gene and the internal transcribed spacer (ITS) region. PNA clamps will be used for 16S amplification to avoid host contamination. The goal is to determine if the microbiomes of the herbarium specimens show similar diversity patterns compared to what is found in silica-dried specimens.



## Climate Change Influence Microbial Community Composition in the Rhizosphere of Boreal Tree Species

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#### Abstract

Climate change is increasing global temperatures and the frequency and severity of droughts in many regions. These anthropogenic stresses pose a significant threat to plant performance in ecotones (transition area between two biological communities) such as the boreal to forest ecotone in Northern Minnesota. The ability of plants to face environmental stresses is partly mitigated by the microbiome inhabiting the soil, rhizosphere, roots, and other plant tissues. However, we have limited understanding on how multiple climate change drivers individually or interactively shape the microbial community structure of plants growing in the temperate-to-boreal forest ecotone. To understand the impact of multiple climate change drivers of plant-microbiome, we collected rhizosphere soil samples from plants growing under ambient, warming, drought, and combined warming-drought treatments from a Boreal Forest Warming in an Ecotone in Danger (B4WarmED) experiment being conducted at two sites in Minnesota. We profiled the bacterial and fungal community structures by performing the amplicon sequencing of 16S rRNA and ITS regions. Our results demonstrate that drought influences the microbial alpha diversity, and warming drives shifts in beta diversity; the results of network analysis including rhizosphere bacteria and fungi showed that under drought and combined warming-drought conditions, microbial communities had more positive correlations than others, co-occurrences network analysis showed that network complexity is more intricate in the north side. Overall, we found that climate change drivers influence rhizosphere microbial communities of temperate-to-boreal ecotone forests.



# Microbiome analysis revealed diverse and distinct microbial communities from field grown peanut nodules

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#### Abstract

Legume nodulation is the powerhouse of the production of biological nitrogen fixation (BNF) where host specific rhizobium strain dominates the nodule microbiome; however, other rhizobial or non-rhizobial inhabitants can also colonize legume nodules. It is unclear how various rhizobial or non-rhizobial colonizers compete or combinedly functions in the nodule microbiome. To investigate these possibilities, we conducted 16S rRNA based nodule microbiome sequencing to characterize microbial communities in two distinct types of field grown peanut nodules (big and small) inoculated with commercial inoculum. We found that microbial communities diverged drastically in the two types of peanut nodules. Core microbial analysis revealed that peanut big nodules inhabited by *Bradyrhizobia sp.*, which is the most abundant genus under phylum protobacteria, dominated (>99%) throughout the plant life cycle. Surprisingly, we observed that in addition to *Bradyrhizobia*, peanut small nodules harbored a diverse set of microbial communities (~31%) that were not present in big nodules. It is worth mentioning that the diverse microbial inhabitants gradually dominated in peanut small nodules during the plant growth stages, which suggested that native microbial communities competed with the commercial inoculum in small nodules only, whereas very negligible or no competition was observed in big nodules. Along with the prediction of KEGG pathway analysis for N and P-cycling related genes and the presence of diverse genera in small nodules, we hypothesize that these microbial communities might play a role for peanut growth and development via growth promotion and/or protecting plants from various biotic and abiotic stress.



## Investigating the Impacts of Drought on Root Microbiome Assembly

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#### Abstract

Global climate conditions influence rapid changes in local environmental conditions and contribute significantly to prolonged ecosystem-level changes. In this work, drought is used as an abiotic stress elicitor to explore the broad impacts of water availability on plant resilience by observing induced shifts in the abundance of microbial groups associated with both natural and agricultural ecosystems. As the microbiome represents a highly tractable model for the analysis of plant resilience, the focus of this work aims to define the convergence of drought and drought tolerance with plant-associated microbial community composition and the subsequent role of the microbiome on plant heath. To begin addressing these broader research questions, a 40-member bacterial synthetic community (SynCom) representative of bacterial taxa commonly found in soil has been used to inoculate wild-type Arabidopsis and a variety of mutant lines to characterize connections between plant genotype and presence of a microbiome impacting plant biomass. These results will be used as a baseline for future drought studies investigating the impact of drought on the assembled microbiome and the potential role of plant immune signaling in response to abiotic stressors. Additionally, preliminary work to confirm a transgenic line of soybeans overexpressing Arabidopsis *NDR1*, previously identified to play a role in immune signaling in response to drought, will provide a potential tool for translating findings in Arabidopsis to an agriculturally relevant system. Taken together, this work lays the foundation for future studies investigating the impacts of drought on microbiome assembly and overall plant resilience.



# **Evaluating Plant-Microbe Interactions: Impacts of metabolic handoffs in the stabilization of synthetic microbial communities.**

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#### Abstract

Plants exude a variety of molecules from their root systems into the surrounding soil that can regulate changes in plantsoil-microbe interactions by affecting the root colonization by symbiotic microbes. Aromatic acids are one of the classes of metabolites exuded by plants and are involved in rhizosphere-microbiome interactions. The objective of this study is to elucidate the role of aromatic acids in metabolic handoffs that strengthen plant-microbe interactions. We profiled root exudates of 12 *Brachypodium distachyon* accessions to identify plants with differential production of exuded aromatic acids. Then, we applied a 17-member synthetic microbial community (SynCom), including bacterial strains that are capable of metabolizing these aromatic acids, to the *Brachypodium* accessions to evaluate the dynamics of root rhizosphere colonization in response to different levels of aromatic acids in root exudates. Results show that microbial communities were driven by plant genotypes with variable composition of aromatic acids and plant compartments. We found that strains with significant degradation of aromatic acids efficiently colonized *Brachypodium* plants and that in the SynCom treatment, total organic carbon present in root exudates decreased. These results indicate that the SynCom has an impact on the metabolic profile of root exudates and aromatic acids produced by plants play an essential role in plant colonization by soil microorganisms. Ongoing work involves creating SynComs with engineered microbes to further evaluate the roles and mechanisms of aromatic acids in rhizosphere microbiome establishment using a RB-TnSeq mutant library of SynCom members and candidate genes related to the microbial degradation of aromatic acids.



## Microbiome-based diagnostic tools for improving primary crop production

<u>Tanja Kostic</u><sup>1</sup>, Livio Antonielli<sup>1</sup>, Johanna Ley<sup>1</sup>, Narciso Martin Quijada<sup>2</sup>, Angela Sessitsch<sup>1</sup> <sup>1</sup>AIT Austrian Institute of Technology GmbH, Tulln, Austria. <sup>2</sup>FFoQSI GmbH, Tulln, Austria

#### Abstract

The application of microbiome-based molecular diagnostics in primary crop production is still underexploited. The main reasons hindering the acceptance of these technologies in the past were high costs and skills/equipment requirements. The emergence of new high-throughput molecular technologies opened new avenues to be explored.

In the scope of the MASTER project (https://www.master-h2020.eu/), we assessed the feasibility and usability of two different microbiome-based diagnostic tools using fodder maize as a model system.

Firstly, we tested the applicability of Oxford Nanopore sequencing technologies for pathogen detection in soils. A range of samples, including ZymoBIOMICS standard, customised mock communities, soil samples (native and spiked with organisms of interest), was used to assess the specificity and sensitivity of the system. In parallel, bioinformatics pipelines were tested and refined to allow efficient and high-quality data processing and taxonomic identification. Our findings showed that the tested method performed qualitatively well in the simple matrices, however, even in this case, quantification potential was sub-optimal. With the increasing level of complexity, both the specificity and quantification potential diminished.

Furthermore, a microbiome-based tool to predict the colonisation efficiency of microbial inoculants was developed. Firstly, the colonisation efficiency of the selected inoculum was assessed, followed by the microbiome analysis. A machine learning model sensitive to different soil types was established and is pending validation in 2022.



# Priming with host-associated bacterial consortia alters microbial community structures in *Arabidopsis* roots and suppresses *Fusarium oxysporum*

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#### Abstract

#### Abstract

The plant-associated microbiota confers beneficial traits to the plant host that promote growth and prevents disease. However, it is not fully understood how the host, and pathogens, modulate the assembly of the associated microbiota. In this work, we investigated the effects of inoculation with bacterial consortia (BC) that had been pre-conditioned with either the host alone or by a combination of host- and pathogen-priming for five successive Arabidopsis generations on the root-associated microbiota and the invading pathogen *Fusarium oxysporum f. sp. mathioli* (FOM). We used 16S rRNA and ITS amplicon sequencing to profile root bacterial and fungal communities, respectively. qPCR was employed for assessment of FOM quantities in shoots of Arabidopsis. Our results showed that host-primed BCs inhibited *F. oxysporum* proliferation in shoots, distinctively affected rhizo-microbial community structures and further enhanced the connectivity of the microbial networks. Likewise, FOM-primed BCs reduced F. oxysporum in the shoots of Arabidopsis and altered microbial networks. Beneficial bacterial families including Rhizobiaceae, Caulobacteriaceae and Azospirillaceae were significant biomarkers in the samples inoculated with host-primed BCs and could potentially be involved in FOM antagonism. Altogether, these findings deepen our understanding of both host and pathogen BCs in modulating host-associated microbiomes and could thus, be exploited in engineering sustainable disease suppressive growth systems.

Keywords: rhizosphere engineering, microbiome inoculation, disease suppression, fungal pathogens, invasion networks.



## Significance of agroecological practices in enhancing soil health and yields for a sustainable tea production in Northern Vietnam

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#### Abstract

Intensive tea cultivation has caused serious soil health degradation globally, including severe soil acidification, metal toxicity, nutrient leaching and imbalance. Conducted accross 20 different tea plantations from 2019-2021, this project examined the impacts of agroecological management practices (mulch and organic fertilizer applications) on tea soil health indicators, yield and quality, and net income of tea farmers in Northern Vietnam. We showed that agroecological practices enhanced soil physicochemical properties by significantly increasing soil OM content and reducing soil acidity. Soil biological properties, including arbuscular mycorrhizal fungi colonization, soil macro and mesofauna abundance, richness and diversity were also significantly higher in agroecological tea soil and root samples than in conventionally managed tea plots. Despite lower tea yield and yield components, agroecological tea management adopters earned around USD 9200 ha/ year more than the non-adopters. To further counter soil acidification and consequently enhance soil health and crop productivity, lime was applied at a rate of 1.5 tons/ha in 6 agroecological tea plantations. Nine months after application, liming significantly increased soil pH by 0.6 units on average. A significant increase of soil macrofauna abundance and diversity were also observed in lime treatments (average of 122 and 89 individuals/m<sup>2</sup> in lime and control plots, respectively). Additional analyses will be conducted on soil microbial communities, tea quality indicators and soil nematodes populations. Our results provide a better understanding of the effects of management practices on soil health and tea productivity and can be used to promote sustainable tea management practices, while increasing farmers' income.



## The effect of maize genotypes, environments, and GXE interactions on maize endophytes

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#### Abstract

Recent studies in the plant-associated microbiome have pointed out that rhizosphere microbiome and endophytes, organisms that live inside plants without causing disease, can benefit their host plant by supplying vital nutrients, manipulating plant hormone levels, and producing secondary metabolites. However, most discoveries focus on an individual plant-microbes relationship and fail to translate into an agricultural benefit in the real world. This simplified view on plant-microbes interaction ignores the fact that plants are under the consistent influence of the environment and change their metabolism to react to environmental stimuli, which indirectly affects the endophyte community. To understand the environmental context of plant-microbe interaction, we sample 25 maize pedigrees in more than 15 locations in 2019 and 2020 and analyze the effect of locations, genotypes, and GXE interaction on the diversity of endophyte and individual taxa. We found the huge GXE and location effect on both alpha diversity of the endophyte community and individual taxa abundance, but a little effect of maize genotypes. We also found the environment factors such as soil pH are associated with community composition.



## **Effect of soil microbiome on plant growth and root-associated microbiomes of canola** (*Brassica napus*.**L**)

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#### Abstract

Under low phosphorus (P) conditions, variation in plant root system architecture (RSA) can play an important role in acquiring P. Roots can modify the rhizosphere to recruit certain soil microbes, making P available for plant uptake. However, it remains unknown whether the root-associated microbiomes and RSA interact and work together to facilitate P acquisition. We conducted rhizobox experiments by growing two canola genotypes with different RSAs in gamma-irradiated and non-irradiated low P soils to test hypotheses that: 1) soil microbiome contributed to plant growth and P uptake, and 2) plant genotypes with diverse RSAs differ in root-associated microbiomes. We found that gamma-irradiation reduced culturable microbes, CO2 respiration rate, and microbial community diversity in soil, while soil nutrients including nitrogen (N), P, organic carbon and sulphur remained stable. Plant growth of both canola genotypes was inhibited in gamma-irradiated relative to non-irradiated soil after 14 days. Meanwhile, colonies were observed only in gamma-irradiated soil, which were identified as members of *Bacilli* and *Penicillium*. Differential abundance analysis showed suppression of specific microbes (e.g., *Chitinophagales* and *Gammatimonadales*) and stimulation of others (e.g., *Bacillales* and *Frankiales*) in gamma-irradiated soil, which disrupted soil health and its microbial homeostasis. This disruption was possibly associated with higher soil CO2 respiration rate and available P, but lower available N. We concluded that soil microbiome is vital for soil health, plant growth and RSA. Further investigations on root microbiome will facilitate our understanding on the impact of soil microbiome, RSA and their interactions on plant P uptake.



#### Plant and Bacterial Inositol Exchange Influences Root Colonization Outcomes

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#### Abstract

Plant rhizospheres host incredibly diverse microbial communities, but only a subset of these microorganisms successfully colonize within root tissue. Considering this, we used meta-transcriptomic networks to identify putative contributing host factors. Our results suggested host transporters for *myo*-inositol, a pre-cursor to integral eukaryotic structural and signaling molecules, may influence endophytic microbiome activity. When we performed bacterial colonization assays using *Ara-bidopsis thaliana* genotypes mutated in various inositol transport and biosynthesis genes, we found significantly reduced colonization levels only for bacterial isolates that metabolize inositol. Additionally, we use both a comparative genomics and molecular genetics approach to understand the importance of microbial inositol catabolism in seedling colonization. Plant *myo*-inositol synthesis and signaling has been implicated in pathogen susceptibility, and microbial inositol usage has been identified as an important virulence factor across a range of hosts. Our findings suggest a more general role for host inositol in microbial root colonization by potentially providing a nutrient source that only a subset of endophytic microbial species to converge on a single metabolite broadly influencing host colonization outcomes. This work expands our knowledge of the potential strategies used by plants and bacteria to moderate symbiotic interactions in a dynamic environment.



#### Microbiota-mediated immunocompetence in Arabidopsis

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#### Abstract

Healthy plants possess a microbiota composed of a multitude of diverse microorganisms which form a variety of relationships with the host plant. To keep pathogenic and opportunistic microbiota members at bay, plants have evolved a twotiered innate immune system capable of recognizing a set of conserved microbial epitopes or specific pathogen-secreted virulence effectors to elicit immune responses and protect against infection. Many individual microbial strains have been shown to ectopically enhance plant immune responses, however the contribution of indigenous microbiota as a whole on plant immunity remains largely unknown. Addressing this question optimally would require a gnotobiotic plant growth system with growth conditions that parallel natural environments. Using a recently developed peat-based gnotobiotic plant growth system that simulates plant growth in soil we found that Arabidopsis grown in the absence of a microbiota are immune compromised and hypersusceptible to bacterial infection compared to plants colonized by a soil-derived microbiota. Further, a 48-member eubiotic synthetic bacterial community isolated from the leaf endosphere of conventionally grown plants was able to restore immunocompetency to inoculated plants. Interestingly, modulation of plant immunocompetence by a microbiota appeared dependent on abiotic plant growth conditions, which indicates a tripartite interaction between the host, microbiota, and environment during microbiota-mediated immune maturation and highlights the importance of environmental factors in when performing microbiota functional studies. Additionally, we found that a 52-member dysbiotic synthetic bacterial community overstimulates immunocompetency in inoculated plants. Overall, our results show a fundamental role of an eubiotic microbiota in the establishment of basal immunity.



## Farm-scale differentiation of active microbial colonizers

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#### Abstract

Microbial movement is important for replenishing lost soil microbial biodiversity and driving plant root colonization, particularly in managed agricultural soils, where microbial diversity and composition can be disrupted. Despite abundant survey-type microbiome data in soils, which are obscured by legacy DNA and microbial dormancy, we do not know how active microbial pools are shaped by local soil properties, agricultural management, and at differing spatial scales. To determine how active microbial colonizers are shaped by spatial scale and environmental conditions, we buried microbial traps (i.e., sterile soil enclosed by permeable mesh membranes) across transects of a forest-adjacent farm and under 16 cover crop treatments in a randomized block design within an organically managed agricultural research farm. Bulk soil and deployed traps were collected for 16S rRNA gene and fungal ITS amplicon sequencing at two time-points to identify early re-colonization and community development. We hypothesized that 1) different cover crop mixtures, either single- or multi-species, would stimulate different portions of the soil microbial pool, and 2) at finer spatial scales, the active (soil-recolonized) microbial pool would be different relative to surrounding bulk soil. We found that bacteria had greater colonization consistency (within-group similarity) among replicate communities. Relative to bacterial colonizers, fungal colonizers shared a greater compositional overlap to sequences from the surrounding local bulk, suggesting that these groups respond to distinct environmental constraints. Understanding how environmental constraints and spatial scales impact microbial recolonization dynamics and community assembly are essential for identifying how farm management can be used to intentionally shape agricultural microbiomes.



# Exploring Microbial Drought Adaptations and Microbially-Mediated Plant Drought Tolerance

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#### Abstract

Drought events are anticipated to increase in frequency and will continue to alter the environment needed for crop production across the globe. Increasing evidence suggests that soil microbes play a large role in plant health and have the ability to adapt rapidly to local environmental conditions. Understanding how soil microbes adapt to water-stress could contribute to the development of sustainable agricultural management strategies. More than 500 bacterial strains from the *Luteibacter* genus were collected from across environments with different precipitation levels. We hypothesize that *Luteibacter* strains from drier sample sites will be more tolerant to osmotic stress, and can improve drought tolerance in plant hosts. Using growth media supplemented with various salt concentrations we measured bacterial osmotic stress tolerance *in vitro*. We selected the 10 most and least tolerant strains (20 strains total), and inoculated these isolates onto one week old *Zea mays* (maize) seedlings growing under simulated drought conditions. Plant biomass and height will be measured to compare the impacts of *Luteibacter* strains on plant drought tolerance. Next, select strains will undergo whole genome sequencing. Using a genome-wide association study (GWAS) approach we will link genetic factors to tolerant microbial phenotypes. This will provide insights into the mechanisms underlying microbial drought adaptations and microbially-mediated plant drought tolerance.



# Microalgae metabolites enhance siderophore producing bacteria and visual appearance of strawberries and almonds with severe IDC

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#### Abstract

In a series of 6 field studies (3 strawberry, 3 almonds), a commercially available microalgae metabolite product (TrueSolum<sup>®</sup>) enhanced microbes associated with siderophore production and iron assimilation. This was supported by improvements in the visual appearance of crops with severe iron deficiency chlorosis. These fields were treated weekly with TrueSolum<sup>®</sup> for approximately 35 days. Soil samples were taken from the rhizosphere pretreatment and 35 days after the initial application of the product and were analyzed for bacterial and fungal changes in the phytobiome using gene sequencing and bioinformatics technology. In all trials, treated plots had higher levels of siderophore producing bacteria compared to the control. Siderophores are strong iron chelators that make iron more available to plants. Key genus proposed to be causing these effects include *Pseudomonas sp*. This is the first report of microalgae metabolites causing beneficial changes in bacterial communities of the soil that influence the availability and movement of key cations, such as iron, which are widely known to be critical components of successful crop production.



## Mycoviruses in Fusarium culmorum identified by high-throughput sequencing

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#### Abstract

Fusarium head blight (FHB) continues to be one of the major problems infecting both wheat seedlings and heads (kernels and spikes), and is considered a disease complex caused by several highly specialized pathogens including F. graminearum and F. culmorum. Biocontrol of FHB is of great economic significance and mycoviruses infecting Fusarium could be a step forward toward the management of FHB disease. While the majority of mycoviruses has been reported as asymptomatic to the host hardly causing any effect on host phenotype, few viruses have been reported to affect fungal growth, also known as hypovirulent mycoviruses. With the objective to investigate hypovirulent mycoviruses, we isolated F. culmorum from wheat (Triticum aestivum) cultivars and screened for mycoviruses. We extracted and sequenced double-stranded RNA (dsRNA). Based on the RNA-dependent RNA polymerase domain analysis, we found several dsRNA but indeed also a few ssRNA mycoviruses. Diverse mycoviruses within the Hypoviridae family (a positive sense ssRNA virus), Unirnaviridae and Partitiviridae, Mymonaviridae, and Aspiviridae families were detected. We will demonstrate the importance of mycoviruses and will discuss their potential role in the biocontrol of FHB.



# Adapting a metagenomic classification tool to reach the resolution required for biosecurity-relevant identification of plant-associated bacteria

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#### Abstract

Plant-associated bacterial strains that belong to the same species can display phenotypic variation relevant to plant biosecurity. For example, different plant pathogen strains that are members of the same species may vary in regards to virulence and host range and be subject to different regulations. To use metagenomics as a tool for the characterization of plant-associated microbiomes for biosecurity purposes, identification of microbiome members at within-species resolution is thus critically important. However, most taxonomic classifiers developed for metagenomics cannot capture diversity within the same species since the species is generally the smallest unit used in taxonomy. We previously developed the Life Identification Number (LIN) concept, which is a hierarchical taxonomy that is independent of traditional taxonomic ranks and uses the individual organism/strain as smallest unit. Phenotypes, such as host range or virulence, can be associated with individual strains or any other within-species groups in the LINbase Web server. Users can then identify query genomes as members of these groups, which we call LINgroups. Here we explored the potential of LINgroups for within-species taxonomic classification of unassembled metagenomes when used in combination with the popular Kraken2 tool. Kraken2 uses NCBI's taxIDs as taxonomy by default. To switch from NCBI's taxIDs to LINgroups, we automated assignment of custom taxIDs to genomes in LINgroups. We successfully used the LINgroup-Kraken2 combination to identify plant pathogens in metagenomic sequences at within-species resolution. Benchmarking is in progress. Challenges and further opportunities for improvement of metagenomics for applications in plant biosecurity will be discussed.



### Bacterial blight R gene expression in high temperature environments

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#### Abstract

Plants experience multiple forms of field stress simultaneously. When this occurs, they must prioritize which form of stress is most important to address when allocating their resources. One rice bacterial blight resistance (R) gene, Xa7, is more effective when exposed to combined high temperature and pathogen stress, whereas other R genes, such as Xa10, lose efficacy at high temperatures. In experiments with a bacterial blight pathogen strains (Xanthomonas oryzae pv. oryzae) that harbor avrXa7 and avrXa10 (effector genes that induce resistance in rice with Xa7 and Xa10, respectively) we compared the impacts of high (35°C day 29°C night) vs low (28°C day vs 21°C night) temperature regimes on bacterial multiplication and spread in planta, and on rice transcriptional profiles. At high temperature regimes, the Xa10 gene lost efficacy, resulting in increased bacterial colonization (spread and multiplication) and more disease (longer lesions) relative to low temperatures, and to Xa7, which was more efficacious at high relative to low temperatures. Interestingly, in vitro studies showed that multiplication of X. oryzae is inhibited by temperatures exceeding 29°C. Our previous work indicated that, at high temperatures, rice without an R gene prioritized biochemical pathways associated with high temperature stress responses, e.g., the ABA pathway. This contrasted with interactions involving rice with Xa7, where the ABA pathway was down regulated. In this presentation, we will further explore high temperature impacts by comparing transcriptional profiles of interactions of high temperatures and rice with Xa7 and Xa10.



# Uncovering exometabolites associated with human pathogen survival in the phyllosphere of diverse lettuce genotypes

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#### Abstract

Contamination of fresh produce from human pathogens continues to pose a risk to food safety. Recent analyses confirmed that disease outbreaks linked with consumption of contaminated leaf greens mostly likely originated in the farm. Thus, understating how these human pathogens survive in the phyllosphere is crucial to design mitigation strategies. Using a panel of 80 lettuce genotypes representing commercial varieties and breeding lines, we discovered that *Escherichia coli* O157:H7 and *Salmonella enterica* persistence in their phyllosphere varies according to the plant genotype, ranging from significant negative to significant positive bacterial net growth over time. These results indicate that there must be genetic and metabolic processes that differ amongst lettuce cultivars, which contribute to O157:H7 and *S. enterica* colonization and persistence in leaves. We also performed a large-scale exometabolomic profiling of the plant spheres where these bacteria reside (i.e., surface phyllosphere and endosphere) to associate chemical composition with bacterial fitness in these environments. Thus far, we were able to identify metabolites that promote or decrease human pathogen survival in different lettuce genotypes. Furthermore, outbreak-associated *S. enterica* strains seem to have differential abilities to utilize nutrients in the leaf exudates and outcompete other members of the microbial community. These findings suggest that a new plant trait (leaf exudate composition) might be used in breeding programs to design plants for the enhanced safety of lettuce grown in California.



## Protists facilitate the establishment of symbiotic bacteria in rhizosphere microbiomes

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#### Abstract

Protists are abundant and impactful components of plant microbiomes. These diverse organisms affect plant health through nutrient cycling and selective predation and can shape the bacterial communities in the phytobiome. However, protists carry their own microbiomes, and the impact of protist-associated bacteria on plant microbiomes is unknown. We hypothesized that protist-associated bacteria may become established or enhanced in the rhizosphere when their protist hosts are inoculated onto a plant. In a growth chamber experiment, maize roots were inoculated with an 18-member consortium of protists isolated from field-grown maize, alone or in combination with a protist-free rhizosphere microbiome. Using high-throughput amplicon sequencing, we found that the addition of the bacterial inoculum did not alter the structure of the protist communities. Conversely, protist inoculation shaped the bacterial rhizosphere microbiomes included bacteria that could establish in the rhizosphere. To test for differences in enhanced bacteria between different protist taxa, we next inoculated maize plants with individual protists in combination with a protist-free bacterial microbiome. We identified 15 bacterial taxa that were greatly enhanced in the rhizosphere protist communities by protists. Different protist cultures introduced different bacterial taxa that could be a function of the distinct protist microbiomes. This research demonstrates that inoculating plants with protists may select for plant-colonizing bacteria, and that protists may be a useful tool for delivering specific bacteria to the rhizosphere.



#### The host-soil-microbiome nexus mediates suppression of bacterial pathogens

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#### Abstract

Conventionally, research on disease-suppressive soils has focussed on open-field cultivation, where shifts in the soil microbiome during continuous monoculture coincide with suppression of soil-borne diseases, as in the take-all decline of wheat. We report soil-microbiome mediated suppression of bacterial pathogens for the first time in horticultural growth media, while focussing on the role of the soil-host-microbiome nexus.

We observed a consistent decline in bacterial blotch diseases of Agaricus bisporus, induced by high pathogen populations in the soil. Changes in the soil microbiome reflected ecological events such as pathogen invasion, pathogen establishment and disease suppression. Disease suppressive genera were found to cluster together in bacterial co-occurence networks, and disease suppression was transferrable via aqueous extracts from suppressive to conducive soils.

Investigation of the host revealed large differences between in-vivo and in-vitro pathogen inoculation, further highlighting the role of the microbiome in disease development. Resistant cultivars actively recruited a beneficial host microbiome from the soil, which was more diverse and comprised many of the same genera involved in disease suppression. Strong trade-offs between productivity and disease suppression in the cropping system were also regulated by the microbiome of the growth media.

We concluded, that the resilience of the host to bacterial diseases is determined not only by genotype, but also by the assembly of- and interactions within- the host-soil-microbiome nexus. This knowledge on microbiome assembly and its dynamics presents new prospects for the design of ecological disease control strategies.



# Sustainable soil management practices determine the root-associated microbiome of soybeans in a 25-year Southern Ontario field trial

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#### Abstract

Intensive agricultural management can exert selective pressure on both soil and plant-associated microbiomes by altering the soil abiotic environment (e.g., organic matter, nutrient content, pH, microclimatic conditions). The goal of this study was to determine the long-term impact of sustainable agricultural strategies (i.e., reduced tillage and crop diversification) on the soybean microbiome. In a 25-year field trial from Ridgetown, ON, Canada, we analyzed two levels of tillage (conventional vs. reduced) and crop rotational diversity (continuous soybean vs. soy-wheat-corn with red clover cover crops). We collected and extracted DNA from bulk soil (0-15 cm), soybean rhizosphere and soybean root endosphere samples. Then, we used amplicon-based high-throughput sequencing (Illumina MiSeg) to analyze prokaryotic (16S rRNA) and fungal (ITS) community structure. Rhizosphere and bulk soil communities presented similar richness of amplicon sequence variants (ASVs), genera and phyla, while endosphere communities were markedly less diverse. Looking at ASV composition, tillage intensity shaped microbial communities in both the rhizosphere and endosphere. Rotational diversity shifted prokaryotic composition only in the endosphere and fungal composition only in the rhizosphere. Neither practice had a clear effect on bulk soil microbial community composition. Despite these results, changes in the relative abundance of microbial genera were clearer in bulk soil, followed by the rhizosphere, with no detectable taxonomic changes within endosphere communities. Our results suggest that sustainable management practices can shape root-associated microbiomes even if the bulk soil microbiome is not significantly affected. However, these changes might not lead to changes in the relative abundance of potentially relevant taxonomic groups.



#### Soil to Seed: The influence of soil microbes on Brassica seed chemistry

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#### Abstract

Could the flavor of plant-based foods be influenced by the microbiome of the soil in which those plants are grown? The chemical content and flavor profile of crops is known to be affected by numerous factors including soil type, plant variety, harvest time, and climate. The role of the soil microbiome in determining crop flavor is less well understood. To test the role soil microbes play in determining flavor chemistry, we grew mustard plants (*Brassica juncea*) in a greenhouse study and manipulated only the microbes applied to the potting soil. We then characterized the chemical composition of mustard seeds produced to test our hypothesis that plants grown with different soil microbiomes produce seeds that have distinct chemical profiles. We focused our chemical analysis glucosinolates, a family of spicy-tasting secondary metabolites that *Brassica* species produce as defense compounds against insects and microbes.

We were able to measure four different glucosinolate-derived compounds in the mustard seeds, with allyl being the most abundant and ubiquitous across samples. We found that differences in soil microbiome community composition correlated with differences in seed glucosinolate chemical composition. Further, we used machine learning methods to identify the specific bacterial and fungal taxa that were most closely associated with shifts in glucosinolate content. This work suggests the potential for manipulating soil microbiomes to influence food flavor or nutritional content.



# Effects of treating rice roots with beneficial microbes on rhizosphere and endosphere microbiome and plant responses to such treatments

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#### Abstract

Dipping rice roots in either *Bacillus subtilis* or *Trichoderma asperellum* at transplanting led to about 20% increase in grain yield as well as reduced development of rice blast in subsistence farming in Africa. We conducted experiments to study possible reasons for such a yield increase resulting from root dipping, particularly in terms of rhizosphere/endosphere microbiome and plant responses. Amplicon sequencing was used to profile rhizosphere and endosphere microbiome after dipping treatments, and meta-transcriptomic sequencing to characterise plant responses. Dipping treatment did not result in any appreciable changes in the overall microbial community structure in both rhizosphere and endosphere. Relative abundance of several specific taxa groups were affected by both *B. subtilis* or *T. asperellum* dipping treatments. However, there is no clear biological relationship of those taxa affected with plant development, except that treatment with *T. asperellum* led to a significant increase of this taxa in both rhizosphere and endosphere. RNA-seq results indicated that expressions of many genes were affected by the dipping treatments; however, large differences between two replicated experiments make it difficult to interpret these differentially expressed genes biologically. New experiments are being conducted to further our understanding on the plant responses to root dipping.



## Microbial structure and metabolic pathway differences in sugarcane and oilcane accessions and their potential implications for bioenergy productivity

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#### Abstract

Sugarcane (*Saccharum* spp. hybrid) holds high potential as a significant source of biomass for biofuel production and can efficiently convert solar energy into stored chemical energy. More recently, oilcane has been metabolically engineered from sugarcane to accomplish hyperaccumulation of lipids in its biomass. Given the reallocation of nutrients in oilcane compared to wild-type sugarcane, we hypothesized that the microbiome composition would differ between these plants. In this study, we characterize the endophytic (leaf, stem, and root) and soil (rhizosphere and bulk) microbiomes of four oilcane accessions and compare them to that of the wild-type sugarcane. Bacterial and fungal DNA was extracted from sugarcane (CP88-1762) and oilcane accessions (17T, 1565, 1566, and 1569) and used to determine the abundance of amplicon sequence variants (ASV). Based on taxonomic profiles, functional genes were inferred to predict potential metabolic pathways. Our results indicate that the bacterial microbiomes of oilcanes are significantly different from sugarcane and specifically highlight that accession 1566 varies significantly from wild-type sugarcane and other oilcane accessions. Core taxa were observed between oilcane accessions and were used to identify taxa that were enriched or depleted in varying oilcane accessions. Taxa related to *Klebsiella* were observed in significantly higher relative abundance in oilcane microbiomes relative to sugarcane. Significant differences in microbial metabolic pathways related to biosynthesis were identified between oilcane variants, specifically 1566 and 1569. Our findings indicate that there are potential taxa candidates that are specific or absent to oilcane accessions and that may impact plant productivity or lipid production.



## *Pseudomonas syringae* **Signaling Molecules Control Bacterial Population Behaviors and** Interactions with the Phyllosphere

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#### Abstract

Bacterial communities rely on quorum sensing mechanisms to detect and respond to communication signals that are directly proportional to cell population density. Bacterial and plant responses to these signaling molecules shapes microbial community behaviors promoting their fitness to the environment they inhabit. Unlike previously thought, we discovered that the phytopathogen *Pseudomonas syringae* pv. tomato (Pst) DC3000 produces the quorum sensing signaling molecule N-acyl homoserine lactone (AHL). Specifically, we functionally characterized putative genes predicted to encode for an AHL synthase (*psyl*) and an AHL receptor (*psyR*). Pst DC3000 only produces the long chain AHL, 3-oxo-C12HSL and the Pst DC3000 *psyl*:/*psyR*<sup>+</sup> double knockout mutant shows hyper motility in culture. The AHL mutant bacterium shows significantly reduced virulence on tomato leaves, where it is unable to induce the formation of necrotic spots typical of infected leaves. Furthermore, using genetics, pharmacological, and omics approaches, we identified a connection between the production of AHL and the phytotoxin coronatine in Pst DC3000. These molecules are secreted on the leaf surface that are essential for Pst DC3000 fitness in this environment and have potential to alter the structure and function of the phyllosphere microbiome.



# Historical climate warming and drought induced shifts in function traits of forest soil microbiome

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#### Abstract

Global climate change, such as warming and drought, can have huge impacts on the structure and functions of soil microbiome with potential feedbacks to various ecosystem functions. However, we have a limited understanding on the impact of multiple climate change drivers on the functional potential of soil microbiome. Here, we used metagenomics to compare the diversity and functions of forest soil microbiome from 2 sites (Cloquet and Ely) in Minnesota, Unites States. The sites are situated at the boreal to temperate forest ecotones with four treatments: 1) control, soil with ambient temperature and precipitation, 2) drought, soil with drought since 2012, 3) warming, soil undergone infrared warming by 3.4 oC above ambient since 2008, and 4) drought-warming, both drought and warming since 2012. Through metagenome profiling, we argue that the micro-environment would be dramatically varied rather than staying stable which are biased towards climatic changes. Our findings indicate that the upregulated functions (such as signal processing, stress protection, and energy consuming) maintain the resilience of soil microbiome against climate change; while the downregulated functions (such as N cycling, CH4 emission, and biofilm protection) are the key trade-offs for overcoming the environmental stress; and those functions (such as amino acid metabolism and carbohydrate metabolism) with general no variances are the buffer pool for microbial living. In brief, our findings provide a new insight in terms of how soil microbiome behave to climate change historically that they are trustful for maintaining basic environmental functions in a long-term run to some extent.



## Linking switchgrass rhizosphere metabolome and microbiome to reveal keystone metabolites under nutrient and moisture limitation

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#### Abstract

Plants exude large quantities of photosynthetically-derived carbon into the rhizosphere. This plant-exuded carbon is composed of a vast diversity of unknown metabolites that could attract or repel soil microorganisms and modulate plant adaptation to environmental stresses. However, it is unclear how collective exudation chemistry and microbial response to these exudates combine with a soil environment to shape the assembly of rhizosphere microbiome and enable plant tolerance to the stressors. To identify links between exudate chemistry, microbial community dynamics, and environmental stress, we grew the bioenergy crop switchgrass in soil, under nutrient limited (-N, -P), moisture limited, nutrient replete (+N, +P, +NP) conditions.

We measured rhizosphere metabolite response to the treatments using LC-MS/MS and linked them to the changes in microbial communities using network analysis. We identified that N-limitation led to the reduced abundance of N-containing metabolites but enriched abundance of aromatic acids and pentoses. We also identified microbial lineages responding to the nutrient's availability. Microbial taxa from Actinobacteria significantly increased in abundance when N was amended. Using network analysis, we identified keystone metabolites that may impact the assembly of the rhizosphere communities, including aromatic acids with a known role in the plant-microbe interactions, and metabolites with a novel function in the rhizosphere, such as serotonin. In further experiments, we confirmed a significant effect of serotonin on switchgrass phenotype and growth of rhizosphere microorganisms. The approach used in this study enabled discovery of the links between identified metabolites, microbiomes, plant phenotypes, and abiotic stresses.



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