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Harnessing the Plant Microbiome Virtual Conference | October 22-24, 2021





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Harnessing the Plant Microbiome

October 22-24, 2021

VIRTUAL CONFERENCE

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Organizers:

Venkatesan Sundaresan University of California, Davis, USA

Maeli Melotto University of California, Davis, USA

Lei Lei Nature Plants, China

Emily White Nature Microbiology, UK

Susan Jones Nature Microbiology, UK

Richard Pattison *Nature Communications,* UK

Joao Duarte Nature Biotechnology, Germany

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Dear conference participants,

It is our great pleasure to welcome you to the "Harnessing the Plant Microbiome" conference, a collaboration between Nature journals and the University of California, Davis.

The potential of harnessing naturally occurring beneficial associations between plants and microbes to enhance crop productivity holds great promise for agriculture. The last decade has seen an explosion of knowledge in the field of plant microbiomes, made possible through technological advances in the characterization and analysis of complex microbial communities. Although the field is still young, major questions such as the mechanisms and interactions underlying plant microbiome assembly and function are beginning to be answered. Moreover, the goals of widespread utilization of plant microbiota for agricultural applications appear to be closer on the horizon. Therefore, we judged that the time was ripe for an international conference to bring together researchers at the forefront of this field to share findings and ideas on current and emerging themes in this rapidly moving research area.

We welcome our keynote speaker Paul Schulze-Lefert, and our invited speakers Yang Bai, Jonathan Conway, Cara Haney, Sheng-Yang He, Talia Karasov, Linda Kinkel, Britt Koskella, Trent Northen, Susannah Tringe, Julia Vorholt, and Maggie Wagner, who had all kindly agreed to participate in the conference when it was still in the planning stages. We also welcome and thank everyone who submitted abstracts, and will be sharing their findings through short talks or poster presentations.

Advances in our understanding of plant microbiomes will be important to help meet the challenges facing an increased need for crop productivity in an era of increased population growth, economic development, and climate change. We hope that this conference will provide a stimulating forum for the exchange of fresh ideas and the forging of productive collaborations in this exciting and promising field of research.

Thank you for joining us.

On behalf of the University of California, Davis, and the editors of the co-organising Nature journals:

Maeli Melotto (University of California, Davis, USA) Venkatesan Sundaresan (University of California, Davis, USA) Joao Duarte (*Nature Biotechnology*, Germany) Susan Jones (*Nature Microbiology*, UK) Lei Lei (*Nature Plants*, China) Richard Pattison (*Nature Communications*, USA) Emily White (*Nature Microbiology*, UK)

We would like to extend our thanks to our sponsors

PLATINUM LEVEL







This work is supported by Agricultural Microbiome in Plant System and Natural Resource Program (grant no. 2021-67013-35810) from the USDA National Institute of Food and Agriculture, and by the Plant Genome Research Program of the National Science Foundation, USA (grant #IOS-2151731).

GOLD LEVEL







SILVER LEVEL







GENERAL INFORMATION

This conference is taking place in **Pacific Daylight Time (PDT)**.

The conference recording will be available ~48 hours after the conference conclusion for registrants to view for up to 60 days.

Scientific Session Protocol

Audio and/or video recording of the scientific sessions is not permitted.

We have set an opt-out social media policy for this meeting. Speakers have been asked to announce at the start of their talk if they do not wish to have attendees tweet, blog or share the content of their talk, or in advance of those sections of their talk that should not be shared. We encourage you to make use of social media using the conference hashtag:

#PlantMicrobiome2021

Abstracts may not be re-printed without permission of the presenter. Poster presentations are considered personal communications, and information in these may not be publicly shared without prior permission of the presenter.

Code of Conduct

Nature Conferences is committed to promoting practices that support diversity and inclusion. We value wide-ranging views, expertise, opinions, backgrounds and experiences. We intend Nature Conferences to be welcoming, safe, collaborative and productive for all attendees, volunteers and staff in both a live and virtual setting.

If you need to report an issue of feeling unsafe or harassed, please email <u>alexandra.glasner@us.nature.com</u> immediately.

Those who are deemed to be acting inappropriately will be expelled from this conference with no refund, at the discretion of the conference organizers.

Internet Access

A strong WiFi connection is key to a successful virtual conference. Please make sure that your WiFi signal is strong enough to support streaming video. If you do happen to lose connectivity, please know that all sessions will be available for viewing ~48 hours after the conference and for the next 60 days.

Our partnered streaming platform Stream Go is most compatible with **Google Chrome**. Please ensure to download Google Chrome to your device before the start of the conference.

Virtual Poster Session

The poster session will be taking place on Saturday, October 23rd from 10:15-11:15 PDT. Posters can be viewed at any time in the **POSTER HALL** tab of the Stream Go site. Some poster presenters have provided a Meeting Room Link, where you can meet and chat to discuss their work during the poster session.

You can also chat with poster presenters – and attendees at any time – with the **Chatbox** which is located on every page of the Stream Go site.

Networking Sessions

We welcome all attendees to take part in our networking sessions on Friday 22 October from 12:00-13:00 PDT and Saturday 23 October from 7:00-8:00 PDT. These sessions are optional but encouraged, as you will be able to interact directly with other attendees, including speakers, sponsors and industry professionals.

Note that there will be links on the **Agenda** page of the Stream Go site for these two events. Join hosts at their tables, interacting via video conference room.

Networking table hosts can be found on pages 13-14 of this program book.

Conference Certificates

If you are in need of a certificate of attendance, registration payment and/or short talk or poster presentation, please email Alexandra Glasner at <u>alexandra.glasner@us.nature.com</u> after the conference concludes.

UCDAVIS OFFICE OF RESEARCH

UC DAVIS STUDENT AWARDS

The UC Davis Office of Research offered a "Virtual Travel" Award to four UC Davis students and post-doctoral fellows presenting their research at this conference.

These are the selected recipients:

Prolonged drought results in enduring compositional changes to the rice root microbiome **Christian Santos Medellin** – SHORT TALK

A proteo-genomics dissection of the Walnut-Xanthomonas arboricola pv juglandis interactome **Renata De Almeida Barbosa** – POSTER PRESENTER

The microbiome of rice without aerenchyma **Aaron Rosenfeld** – POSTER PRESENTER

The effects of Urea fertilizer on methanogenesis and related upstream metabolic processes in rice-associated microbiomes **Zach Liechty** – POSTER PRESENTER

HARNESSING THE PLANT MICROBIOME

University of California, Davis Davis, California, USA

October 22-24, 2021

*Times below are in Pacific Daylight Time (PDT) and on a 24-hour clock // London (BST) +8 hours ahead

Day 1: Friday, October 22, 2021

8:00 - 8:15	Welcome remarks Venkatesan Sundaresan (University of California, Davis, USA)
8:15 – 9:00	Keynote: Assembly and host specificity in the bacterial root microbiota Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Germany)
SESSION I:	Cross-kingdom interactions <u>Moderator</u> : Susan Jones (<i>Nature Microbiology</i> , UK)
9:00 – 9:30	Mechanisms, regulation, and ecology of bacterial auxin degradation in the complex root microbiome Jonathan Conway (Princeton University, USA)
9:30 - 9:45	Short talk : <i>Plant immune system activation is necessary for efficient interaction with beneficial bacteria</i> Elhanan Tzipilevich (Duke University, USA)
9:45 - 10:15	From theory to reality: can we build a functional plant microbiome? Britt Koskella (University of California, Berkeley, USA)
10:15 - 10:45	5 BREAK
SESSION II:	Molecular signals and communication <u>Moderator</u> : Richard Pattison (<i>Nature Communications</i> , USA)
10:45 - 11:15	Molecular dissection of dysbiosis in plants Sheng Yang He (Duke University, USA)
11:15 - 11:30	Short talk: Effect of strigolactones on microbiome recruitment in rice Bora Kim (University of Amsterdam, Netherlands)
11:30 - 12:00	<i>Mechanisms in host regulation of the rhizosphere microbiome</i> Cara Haney (University of British Columbia, Canada)
12:00	End of Day 1

12:00 - 13:00	OPTIONAL NETWORKING SESSION - interact directly with other	
	attendees, speakers, sponsors, industry professionals and Nature	
	journal editors during the table networking session - see pages 13-14 for	
	details.	

Day 2: Saturday, October 23, 2021

7:00 - 8:00 OPTIONAL NETWORKING SESSION – interact directly with other attendees, speakers or take part in a satellite workshop during the table networking session - see pages 13-14 for details.

SESSION III:	Plant microbial ecology <u>Moderator</u> : Emily White (<i>Nature Microbiology</i> , UK)
8:00 - 8:30	The leaf microbiota: responses and impacts on plants Julia Vorholt (ETH Zürich, Switzerland)
8:30 - 9:00	Diffuse interactions: Competitive and coevolutionary interactions as drivers of microbiome functions Linda Kinkel (University of Minnesota, USA)
9:00 - 9:15	Short talk: Prolonged drought results in enduring compositional changes to the rice root microbiome Christian Santos Medellin (University of California, Davis, USA)
9:15 - 9:45	BREAK
9:45 - 10:15	Host and environmental drivers of phyllosphere composition across Europe Talia Karasov (The University of Utah, USA)
10:15 - 11:1	5 POSTER SESSION
11:15 - 11:3	0 BREAK

SESSION IV:	Microbiome analytics <u>Moderator</u> : Venkatesan Sundaresan (University of California, Davis, USA)
11:30 - 12:00	Sorghum root microbiome dynamics under nutrient-limited conditions Susannah Tringe (US Department of Energy Joint Genome Institute, USA)
12:00 - 12:15	Short talk: Plants speak chemistry: Steps to unveil the hidden lexicon of crop holobionts for sustainable agriculture Claudio Screpanti (Syngenta Crop Protection, Switzerland)
12:15 - 12:45	Deconstructing rhizosphere dynamics using fabricated ecosystems Trent Northen (Lawrence Berkeley National Laboratory, USA)
12:45	End of Day 2

Day 3: Sunday, October 24, 2021

SESSION V:	Biotechnology and agricultural management Moderator: Lei Lei (<i>Nature Plants,</i> USA)
9:00 - 9:30	The function and genomic resource of root microbiome revealed by field- grown crops and cultivated bacteria Yang Bai (Institute of Genetics and Development Biology, China)
9:30 - 9:45	Short talk : Cytokinin drives assembly of the phyllosphere microbiome through structural and chemical cues Rupali Gupta (Volcani Institute, Israel)
9:45 - 10:00	Short talk : <i>Precursor-directed activation of microbial volatiles to suppress seed germination of the plant parasitic weed Striga hermonthica</i> Raul Masteling (Leiden University, Netherlands)
10:00 - 10:15	Short talk : Seasonality and shelf life are main drivers of the microbiome and E. coliO157:H7 survival on cold-stored lettuce cultivated in a major production area in California Maria Brandl (USDA Agricultural Research Service, USA)
10:15 - 10:45	Hybridization, heterosis, and the maize microbiome Maggie Wagner (University of Kansas, USA)
10:45 - 11:00	Closing remarks Maeli Melotto (University of California, Davis, USA)
11:00	End of conference

NETWORKING SESSION TABLES

Friday, 22 October – 12:00-13:00 PDT

TABLE	HOSTS
Jonatha	an Conway, Speaker
Cara Ha	iney, Speaker
Linda K	inkel, Speaker
Trent N	orthen, Speaker
Susann	ah Tringe, Speaker
Julia Vo	rholt, Speaker
Maggie	Wagner, Speaker
Britt Ko	skella, Speaker
Richard	Pattison, Editor, Nature Communications
Susan J	ones, Editor, Nature Microbiology
NIFA-N	SF-PBI Funding Opportunities
Hosts:	Ann Lichens-Park (NIFA) Kari Perez (NIFA) Michael Mishkind (NSF)
NSF: IO	S & MCB Funding Opportunities
Hosts:	Diane Okamuro (NSF) Steve DiFazio (NSF) Cliff Weil (NSF)
AgBiom	ie
Host:	Jun Goh, Brant Johnson
BASF	
Hosts:	Suri Jiao Emir Islamovic Benjamin Knudsen
Bayer	
Host:	Thomas Varghese
Novozy	mes, Inc.
Host:	Laura Vann
Syngen	ta
Host:	Claudio Screpanti

Saturday, 23 October – 7:00-8:00 PDT

TABLE HOSTS

Yang Bai, Speaker

Lei Lei, Editor, Nature Plants

Emily White, Editor, Nature Microbiology

Joao Duarte, Editor, Nature Biotechnology

Satellite Workshop: "Microbial contaminants in circular plant-derived food and feed production systems"

Hosts: Leo van Overbeek Nicola Holden

*This table will redirect you to an outside link to take part in the workshop.

SPEAKER BIOS (IN ALPHABETICAL ORDER BY LAST NAME)

Yang Bai

Institute of Genetics and Developmental Biology, China



Yang Bai is a principle investigator in the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China. He received a Ph.D from University of Cologne for plant developmental biology in 2010. From 2011 to 2016, he did a Postdoc at the Max Planck Institute for Plant Breeding Research in the lab of Professor Paul Schulze-Lefert. There, he worked on the highthroughput bacterial isolation and reconstitution of Arabidopsis root microbiota. In May 2016, he built up a research group in Beijing on the function and mechanism of plant-microbiome interactions in crops and Arabidopsis.

Maria Brandl – SHORT TALK PRESENTER USDA Agricultural Research Service, USA

Maria Brandl received her PhD in Plant Pathology at the University of California, Berkeley. Dr. Brandl is a research microbiologist in the Produce Safety and Microbiology Research Unit at the US Department of Agriculture, Agricultural Research Service. She was the Lead Scientist of the Human Pathogen-Plant Interactions Project at the ARS, WRRC for 15 years. Her research focuses on the ecology of Shiga-toxin producing *E. coli* and *Salmonella enterica* in the pre- and post-harvest phyllosphere of crops associated with outbreaks of foodborne disease.



Jonathan Conway

Princeton University, USA

Jonathan Conway is an assistant professor in the Department of Chemical and Biological Engineering at Princeton University. A native of Lancaster County, PA, Jonathan attended the University of Notre Dame for his undergraduate degree in chemical engineering. Jonathan earned his Ph.D. in chemical engineering from North Carolina State University under the direction of Dr. Robert Kelly. He was subsequently a postdoctoral a researcher in the lab of Dr. Jeff Dangl at the University of North Carolina Chapel Hill. The Conway Lab at Princeton is focused on mechanistically defining and engineering plant-microbe interactions at plantmicrobe interfaces.



Rupali Gupta – SHORT TALK PRESENTER Volcani Institute, Israel



Rupali Gupta is working as a Post Doctoral Researcher at the Plant Protection Institute, Agricultural Research Organization-Volcani Institute, Israel. She obtained her Master's degree in Microbiology and Doctorate in Biological Sciences (Microbiology) from the Academy of Scientific & Innovative Research (AcSIR), CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), India. Rupali has been awarded with the Indian Council of Agricultural Research-Senior Research Fellowship, SERB-National Postdoctoral Fellowship of the Department of Science and Technology (DST), Government of India, and ARO Postdoctoral Fellowship (2018) of the Volcani

Institute, Israel. With a Doctorate in Biological Sciences and a Masters in Microbiology, Rupali investigates how the plant immune system protects plants against microbial pathogens, and how beneficial microbes associated with plants stimulate plant growth and health. Her current research is focused on investigating the effects of plant structure and development on plant pathogen interactions and crosstalk between plant defense hormones. With this research, Rupali aims to contribute to societal challenges such as food security and sustainable agriculture

Cara Haney University of British Columbia, Canada



Dr. Cara Haney is an associate professor and Canada Research Chair in plant-microbiome interactions in the Department of Microbiology & Immunology at the University of British Columbia. Prior to joining the UBC faculty in 2016, she was a postdoctoral fellow at Harvard Medical School. Her lab uses high throughput screening combined with genetic and genomic approaches to identify the genetic basis of beneficial traits in plant-microbiome interactions. Her research focuses on elucidating basic mechanisms in host-microbiome interactions. and in finding sustainable solutions for agronomically important challenges in the face of a changing climate.

Sheng-Yang He Duke University, USA

Sheng-Yang He is Benjamin Ε. Powell Distinguished Professor at Duke University and an Investigator at Howard Hughes Medical institute. His lab uses the plant-Pseudomonas syringae pathosystems to discover some of the basic principles that govern bacterial pathogenesis and disease susceptibility in plants. Results from his lab have led to original insights into important cellular processes governing plant-microbe interactions, including plant immunity, bacterial virulence, jasmonate signaling and stomatal defense. Recent research in his lab begins to shed light on how climate conditions influence disease development and how plants control microbiota homeostasis for



health. Dr. He received his Bachelor's and Master's degrees from Zhejiang (Agricultural) University, China, and a PhD degree from Cornell University, USA. He is a fellow of the American Association for the Advancement of Science and a member of the United States National Academy of Sciences.

Talia Karasov University of Utah, USA



Born and raised in Madison, Wisconsin USA, Talia first became interested in evolutionary genetics as an undergraduate working with Dmitri Petrov at Stanford University. As a graduate student with Joy Bergelson at the University of Chicago, she studied how interactions with microbes influence the evolution of plant immune systems. She continued this work with Detlef Weigel at the Max Planck Institute for Developmental Biology in Germany, focusing on how host immune diversity influences pathogen evolution. In Fall 2020 she moved to the University of Utah where she is an Assistant Professor in the School of Biological Sciences.

Bora Kim – SHORT TALK PRESENTER University of Amsterdam, Netherlands

Bora Kim is a PhD student at Plant Hormone Biology Group, University of Amsterdam. She is intrigued as to uncovering a story of rhizosphere, in particular, which plant chemical signaling molecules mediate interactions with microbes and how the responses of microbes can affect plant health. Her ultimate research aim is to find hidden patterns underlying events in the rhizosphere through omics approach and to predict how nature works.



Linda Kinkel

University of Minnesota, USA

Linda Kinkel is a Professor of Plant Pathology at the University of Minnesota. She completed graduate studies at the UW-Madison (M.S. and Ph.D. in Plant Pathology, M.S. in Biometry), and Post-doctoral research at the University of California-Berkeley. Dr. Kinkel's research focuses on the ecology and evolutionary biology of plantassociated microbes in native and agricultural habitats. She is especially interested in managing indigenous populations of beneficial bacteria to enhance plant health and productivity. Her team's current work integrates culture-based and 'omics analyses of soil and endophytic microbial populations in relation to antibiotic production, disease suppression, plant growth promotion,



and soil carbon. Their work has generated substantial insights into the significant roles of microbial species interactions in mediating the functional capacities of plant microbiomes.

Britt Koskella

University of California, Berkeley, USA



Britt Koskella is an Associate Professor in Integrative Biology at the University of California, Berkeley. Her work explores the importance of the bacteria and viruses making up the microbiome in shaping plant health, ecology, and evolution. She received her BA from the University of Virginia in 2001 and her PhD from Indiana University in 2008, and subsequently held postdoctoral and independent research fellowships in both the US (funded by the NSF) and UK (funded by NERC) at Oxford University and the University of Exeter. Her work is focused on understanding how bacteriophage viruses shape bacterial evolution, microbiome diversity, and ultimately the health of host organisms. She combines laboratory experimental evolution with studies of

natural diversity to determine when and how phages impact microbial diversity, focusing primarily on the plant phyllosphere (above ground). More recently she has been exploring both how and why the microbiome associated with plants shapes pathogen establishment and disease progression, and has demonstrated that plant microbiomes can be selected upon to be better adapted to their hosts. Her studies have focused on long-lived trees as well as short-lived and agriculturally relevant systems, and have cemented both the important role of phyllosphere microbiomes in plant health and the need to include bacteria-phage interactions as we move towards predicting microbiome complexity and stability. Her work has also highlighted key open questions in the field that must be addressed in order to translate experimental findings into application, for example to improve agricultural sustainability.

Raul Masteling – SHORT TALK PRESENTER Leiden University, Netherlands

Raul Masteling is a joint PhD candidate at the Netherlands Institute of Ecology (NIOO-KNAW) and the University of Leiden (Netherlands). After getting his MSc in Plant Biotechnology at Wageningen Universitv and Research (Netherlands), Raul is pursuing a PhD in the context of the PROMISE consortium. Here, he is following his passion for nature-based solutions to agricultural challenges by investigating how soil microbiomes can be harnessed for the control of agricultural pests, particularly Striga hermonthica, in a sustainable and cost-effective manner.



Trent Northen

LBL Biosciences Berkeley, USA



Dr. Northen obtained his BS in Chemical Engineering at the University of California Santa Barbara followed by a PhD in Chemistry and Biochemistry from Arizona State University with Prof. Neal Woodbury. He then did a Post-Doctoral Fellow at the Scripps Research Institute in mass spectrometry and metabolomics with Prof. Gary Siuzdak. Dr. Northen started his lab at Berkeley Lab in 2008 and is currently Deputy Division Director and a Senior Scientist within the Environmental Genomics and Systems Biology Division and is the Metabolomics Program Lead at the DOE Joint Genome Institute. He has received numerous awards including a DOE Early Career Award, two R&D100 awards, and

was awarded a Presidential Award for Science and Engineering (PECASE) by President Obama. His research has resulted in over 25 patent applications and >150 peer reviewed publications including numerous papers in influential, peer-reviewed journals.

Christian Santos Medellin – SHORT TALK PRESENTER University of California, Davis, USA



Christian Santos-Medellín received his B.Sc. in Biology at the Universidad Nacional Autónoma de México, where he studied the genomic diversity of host-associated and environmental Pseudomonas aeruginosa. He moved to the University of California, Davis to earn a Ph.D. in Genetics and Genomics under the supervision Dr. Venkatesan of Sundaresan. For his dissertation project, he studied the compositional dynamics and functional implications of the bacterial and archaeal communities associated with rice plants. He is currently a postdoctoral scholar working with Dr. Joanne Emerson to characterize the impact of wet-up and drydown cycles on the viral communities inhabiting Mediterranean grassland soils.

Claudio Screpanti – SHORT TALK PRESENTER Syngenta Crop Protection, Switzerland

Claudio Screpanti is an agronomist working in Syngenta R&D in Switzerland. He obtained his PhD in agronomy from the University of Bologna, Italy in 2003. Claudio joined Syngenta R&D in 2005. covering differentscientific roles always in relation to soil biology. In his current role as Syngenta Fellow, Claudio leads the Soil Health Centre in Stein (Switzerland). The main research activities concern the behavior and effects of new small molecules in the soil-crop systems. The final goal is to support the discovery and development of new and more sustainable crop protection solutions.



Paul Schulze-Lefert

Max Planck Institute for Plant Breeding Research, Germany

Paul Schulze-Lefert is director of the Department of Plant-Microbe Interactions at the Max Planck Institute for Plant Breeding Research in Cologne, Germany. Previously, he held senior positions at the University of Aachen and at the Sainsbury Laboratory of the John Innes Centre in the UK. His research focuses on the plant innate immune system and the plant microbiota. In recent years, his laboratory has contributed to the development of plant microbiota science as a new field of research. His main goal is to define the molecular principles underlying plantassociated microbial communities and their beneficial services to the host usina reductionist approaches.



Susannah Tringe

US Department of Energy Joint Genome Institute, USA



Susannah Green Tringe is the Director of the Environmental Genomics and Systems Biology division at Lawrence Berkeley National Laboratory. Her research focuses on using nucleic acid sequence data to study communities of microbes from diverse environmental niches and understand their assembly and function. Her major research interests relate to how microbes interact with plants to affect growth, health and stress resistance as well as how microbes influence greenhouse gas uptake and release in wetlands and agricultural systems.

Susannah received her undergraduate degree in

Physics from Harvard University then went on to a Ph.D. in Biophysics from Stanford University. She joined Berkeley Lab as a postdoc at the Joint Genome Institute in 2003. There she developed techniques for using DNA sequence data for comparative analysis of whole microbial communities, rather than individual organisms. She has since used and expanded on these methods to demonstrate that microbes interact with each other and with their environment to perform functions that can potentially be harnessed for improved environmental and agricultural outcomes.

Elhanan Tzipilevich – SHORT TALK PRESENTER Duke University, USA

Elhanan Tzipilevich is a postdoc at the lab of Prof. Philip Benfey at Duke University.

His research is focused on root development in the face of microbial interaction.



Julia Vorholt ETH Zurich, Switerland



Julia Vorholt studied biology at the Universities of Bonn and Marburg, Germany. During her PhD, she worked on the biochemistry of methanogenic archaea under the supervision of Prof. Dr. R. K. Thauer at the Max-Planck-Institute for terrestrial Microbiology. She then moved to the laboratory of M. E. Lidstrom in Seattle, USA, as a postdoctoral fellow and subsequently returned to the MPI Marburg, where she established a research group on the biochemistry of methylotrophic bacteria. She then led an independent research group at the Centre National de la Recherche Scientifique (CNRS) in Toulouse, France, where she initiated work on plant microbiomes and

microbial interactions. In 2006, she was appointed associate professor at ETH Zurich. Since 2012, she has been a full professor at the Institute of Microbiology at ETH Zurich. Julia is co-director of the Swiss National Center of Competence in Research (NCCR) Microbiomes. She is a member of the German National Academy of Sciences Leopoldina, the European Academy of Microbiology and the European Molecular Biology Organization (EMBO) and received two ERC Advanced Grants.

Maggie Wagner

University of Kansas, USA

Dr. Maggie Wagner earned her B.S. in Plant Biology at the University of Michigan, followed by a Ph.D. in Genetics & Genomics at Duke University. Her dissertation research focused on ecological evolutionary the causes and consequences of phenotypic plasticity in a wild perennial mustard species, and she developed a strong interest in plant-associated microbiomes both as a critical component of the plant's environment and as an "extended phenotype" of the plant host. She was awarded an NSF Plant Genome Postdoctoral Fellowship to investigate how modern breeding affects the composition and function of the maize microbiome, while based at North Carolina State University. In 2019



she established her lab in the Department of Ecology and Evolutionary Biology and the Kansas Biological Survey at the University of Kansas, where she continues to work on the complex interplay between plant genotype, phenotype, and microbiome.

SPEAKER ABSTRACTS

(IN PROGRAM ORDER)

KEYNOTE

ASSEMBLY AND HOST SPECIFICITY IN THE BACTERIAL ROOT MICROBIOTA

<u>Paul Schulze-Lefert</u>¹, Kathrin Wippel¹, Ruben Garrido-Oter¹, Ka-Wai Ma¹, Jana Ordon¹, Yulong Niu¹, Ke Tao², Simona Radutoiu²

¹Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany. E-mail: <u>schlef@mpipz.mpg.de</u>; ²Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Aarhus, Denmark

Healthy plants in nature are colonized by multi-kingdom microbial communities, termed the plant microbiota, which promote plant growth and health. Our work focuses on the bacterial root microbiota. We have established culture collections of the root microbiota of the crucifer Arabidopsis thaliana and the legume Lotus japonicus, allowing us to purify the majority of bacterial taxa associated with healthy roots. Using taxonomically paired synthetic communities from L. japonicus and A. thaliana in a multi-species gnotobiotic system, we detect signatures of host preference among commensal bacteria in a community context, but not in monoassociations. We find that host preference is prevalent in commensal bacteria from diverse taxonomic groups and that this trait is directly linked to their invasiveness into standing rootassociated communities. In another line of research, we are investigating the mechanisms underlying the assembly of the bacterial microbiota in roots. We find that taxonomically diverse root bacterial commensals suppress A. thaliana immune responses triggered by immunestimulating microbial elicitors or damage-associated molecular patterns. Microbiota reconstitution experiments with defined bacterial communities suggest that immune-suppressive and non-suppressive root commensals modulate host susceptibility to pathogens by either eliciting or dampening MAMP-triggered responses, respectively. This interplay appears to buffer the plant immune system against pathogen perturbation and defense-associated growth inhibition, ultimately leading to commensal-host homeostasis. I will describe experiments aimed at identifying bacterial and host factors that are needed for microbe-host homeostasis.

MECHANISMS, REGULATION, AND ECOLOGY OF BACTERIAL AUXIN DEGRADATION IN THE COMPLEX ROOT MICROBIOME

Jonathan M. Conway^{1,2,3}, Isai Salas-González^{2,3}, Omri M. Finkel^{2,3,4}, William G. Walton⁵, Matthew R. Redinbo⁵, Jeffery L. Dangl^{2,3}

¹ Present address: Department of Chemical and Biological Engineering, Princeton University, Princeton, New Jersey, USA; ²Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; ³Howard Hughes Medical Institute, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; ⁴ Present address: Department of Plant and Environmental Sciences, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel; ⁵Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Chemical signaling in the plant microbiome can have drastic effects on microbial community structure, and on host growth and development. Previously, we demonstrated that the auxin metabolic signal interference performed by the bacterial genus Variovorax via a novel auxin degradation locus was essential for maintaining stereotypic root development in an ecologicallyrelevant bacterial synthetic community. Here, we dissect the Variovorax auxin degradation locus to define the genes necessary and sufficient for indole-3-acetic acid (IAA) degradation and signal interference. We determine the crystal structures and binding properties of the locus's MarRfamily repressor with IAA and other auxins. We identify auxin-degradation loci across the bacterial tree of life and define two distinct types based on gene content and metabolic products: *iac*-like and *Variovorax*-like. We solve the structures of MarRs from representatives of each auxin degradation locus type, establishing that each have distinct IAA binding pockets. Comparison of representative IAA degrading strains from diverse bacterial genera show that while all degrade IAA, only strains containing a Variovorax-like auxin degradation locus interfere with auxin signaling in a complex synthetic community context. This suggests that Variovorax-like locus containing strains play a key ecological role in modulating auxins in the plant microbiome.

SHORT TALK

PLANT IMMUNE SYSTEM ACTIVATION IS NECESSARY FOR EFFICIENT INTERACTION WITH BENEFICIAL BACTERIA

Elhanan Tzipilevich, Philip Benfey

Department of Biology and Howard Hughes Medical Institute, Duke University, Durham, North Carolina, USA

Plants continuously monitor the presence of microorganisms through their immune system to establish an adaptive response. Unlike immune recognition of pathogenic bacteria, mechanisms by which beneficial bacteria interact with the plant immune system are not well understood. Analysis of colonization of *Arabidopsis thaliana* by auxin producing beneficial bacteria revealed that activation of the plant immune system is necessary for efficient bacterial colonization and auxin secretion. A feedback loop is established in which bacterial colonization triggers an immune reaction and production of reactive oxygen species, which, in turn, stimulate auxin production by the bacteria. Auxin promotes bacterial survival and efficient root colonization, allowing the bacteria to inhibit fungal infection and promote plant health.

FROM THEORY TO REALITY: CAN WE BUILD A FUNCTIONAL PLANT MICROBIOME?

Britt Koskella

University of California, Berkeley, Berkeley, California, USA

There is great interest in manipulating, supplementing, or re-seeding the plant microbiome. Given the wealth of data accumulating on plant microbiomes above and below ground from natural and agricultural systems, there is reason to be optimistic that we might be close to achieving this goal. However, the ecological complexity that exists from within microbiomes to between the host and its microbiome to the larger community context in which the plants resides makes translating correlation data from the field or experimental manipulations from the greenhouse a grand challenge. In this talk I will discuss how we can use existing theory and data to build predictions about if and how plant microbiome manipulations will work, and then raise a number of open questions that must be addressed before the full potential of this approach can be realized.

MOLECULAR DISSECTION OF DYSBIOSIS IN PLANTS

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The aboveground parts of terrestrial plants (collectively called phyllosphere) represent one of the most abundant habitats for microbiota colonization on Earth. How plants control phyllosphere microbiota to ensure plant health is not well understood. We recently found that the Arabidopsis quadruple mutants (min7 fls2 erf cerk1; mfec1 hereinafter and min7 bak1 bbk1 cerk1; mbbc hereinafter) simultaneously defective in pattern-triggered immunity and the MIN7 vesicle traffic pathway display leaf tissue damages associated with dysbiosis. Dysbiosis was associated with global alterations in the composition and level of the phyllosphere microbiota, bearing some cross-kingdom resemblance to what occurs in dysbiosis in humans. Bacterial community transplantation experiments using potting mix-based gnotobiotic systems showed a causal role of a properly assembled leaf bacterial community in phyllosphere health. These results highlight critical roles of pattern-triggered immunity and the MIN vesicle trafficking pathway in regulating phyllosphere microbiome homeostasis. We are currently taking a genetic approach to identify additional Arabidopsis mutants, hoping to discover other plant metabolic and signaling pathways that might be involved in regulating phyllosphere microbiome. A better understanding of how plants prevent dysbiosis could make a unique contribution to the ongoing effort to decipher plant-microbiome interactions and has significant basic and applied implications in plant sciences.

SHORT TALK

EFFECT OF STRIGOLACTONES ON MICROBIOME RECRUITMENT IN RICE

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Strigolactones are endogenous plant hormones regulating plant development and are exuded into the rhizosphere when plants experience nutrient deficiency. There, they promote the mutualistic association of plants with arbuscular mycorrhizal fungi that help the plant with the uptake of nutrients from the soil. This shows that plants actively establish – through the exudation of strigolactones - mutualistic interactions with microbes to overcome inadequate nutrition. The signaling function of strigolactones could possibly extend to other microbial partners, but the effect of strigolactones on the global root and rhizosphere microbiome remains poorly understood. Therefore, we analyzed the bacterial and fungal microbial communities of 16 rice genotypes differing in their root strigolactone exudation. Using multivariate analyses, distinctive differences in the microbiome composition were uncovered depending on strigolactone exudation. Moreover, the results of regression modeling showed that structural differences in the exuded strigolactones affected different sets of microbes. In particular, orobanchol was linked to the relative abundance of Burkholderia-Caballeronia-Paraburkholderia and Acidobacteria that potentially solubilize phosphate, while 4-deoxyorobanchol was associated with the genera Dyella and *Umbelopsis*. With this research, we provide new insight into the role of strigolactones in the interplay between plants and microbes in the rhizosphere.

MECHANISMS IN HOST REGULATION OF THE RHIZOSPHERE MICROBIOME

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Below ground, plant roots associate with complex communities of microbes that positively affect plant traits including resistance to biotic and abiotic stresses. By re-screening a collection of *Arabidopsis* mutants affecting root immunity and hormone crosstalk, we identified several plant mutants with altered *Pseudomonas fluorescens* levels without phylum-level dysbiosis. Among these mutants, we identified a new mutant allele of *FERONIA* (*FER*) receptor kinase (*fer-8*) with increased levels of rhizosphere *P. fluorescens*. Using microbiome transplant experiments, we found that the *fer-8* microbiome was beneficial and promoted plant growth. We found that the *fer-8* mutant has reduced basal levels of reactive oxygen species (ROS) in roots and that mutants deficient in NADPH oxidase showed elevated rhizosphere pseudomonads. The addition of RALF23 peptides, a FER ligand, was sufficient to enrich *P. fluorescens*. This work shows that mutants affecting root immunity-hormone cross talk may also affect rhizosphere microbiome structure and function.

THE LEAF MICROBIOTA: RESPONSES AND IMPACTS ON PLANTS

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Microorganisms occur in virtually all environments, are an integral part of complex multicellular organisms such as plants, animals and humans, and play a critical role in their health. We established reductionist approaches to unravel the inherent complexity of microbial interactions and plant-microbiota interactions in situ using the model plant Arabidopsis and gnotobiotic systems. We isolated bacterial strains that represent the majority of species living in the phyllosphere of Arabidopsis to serve as a basis for studying the structure and function of plant microbiomes. We conducted a systematic analysis of plant protection capabilities of the strain collection against the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000. Protection against the pathogen varied, with about 10% of leaf microbiota strains providing full protection and 10% showing intermediate levels of protection. The most protective strains were distributed across different taxonomic groups. Synthetic community experiments revealed additive effects of strains but also that a single strain can confer full protection in a community context. We exemplify that multiple mechanisms contribute to plant protection and include both bacteriabacteria interactions and indirect protection via the plant. By investigating the plant response to members of its microbiota, we identified a molecular response, which is induced by the presence of a majority of strains and include the most differentially regulated genes across treatments. Our results suggest that these genes are part of a defense adaptation strategy that is consistently elicited by diverse strains from various phyla and contributes to host protection.

DIFFUSE INTERACTIONS: COMPETITIVE AND COEVOLUTIONARY INTERACTIONS AS DRIVERS OF MICROBIOME FUNCTIONS

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Plant-associated microbiomes play critical roles in mediating crop productivity, but our understanding of the factors that mold the composition, diversity, and functional capacities of plant microbiomes remains limited. We studied the effects of management approaches that target plant productivity and nutrition on the composition and functional characteristics of endophytic and soil microbiomes. Specifically, we characterized the effects of effects of NPK amendment on foliar endophytic bacterial and fungal community composition and phenotypes, and the effects of soil carbon amendments on microbiome composition and functional capacities in soil. We used amplicon sequencing and culture-based phenotypic analyses for characterizing microbiome composition and functional capacities.

Soil amendment with NPK had significant effects on the composition and phenotypic characteristics of endophytic fungal, but not bacterial, communities. Fungal populations in the NPK-amended plants had reduced resource competitive abilities, but greater antagonistic capacities than fungi in non-amended plants.

Carbon amendments to soil also had significant impacts on soil microbiomes. Both antibiotic inhibitory phenotypes and nutrient competition were increased following soil carbon amendment, though changes varied with the specific carbon type, carbon complexity (single vs. multiple carbons), and carbon quantity.

Across distinct experimental platforms, plant microbiome composition and phenotypes changed in response to nutrient amendments. Notably, nutrient inputs altered the apparent competitive abilities of bacterial and fungal populations for resources, as well as their inhibitory phenotypes, suggesting a critical role for nutrient-mediated competitive and coevolutionary interactions in microbiome assembly. Understanding the impacts of management-induced changes in plant and soil microbiomes may help to identify fundamental principles of microbiome nutrition in agriculture, and stimulate the development of strategies for active management of microbiome functions to support crop productivity.

SHORT TALK

PROLONGED DROUGHT RESULTS IN ENDURING COMPOSITIONAL CHANGES TO THE RICE ROOT MICROBIOME

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As the largest cause of crop loss around the world, drought represents a major threat to food security. Among the efforts to improve crop resilience, plant-microbe symbioses have recently emerged as a complementary and sustainable alternative to traditional breeding, stressing the importance of in-depth investigations of root microbiome responses to drought cycles. While several studies have dissected the compositional shifts undergone during drought, the long-term effects on root microbiomes are not well understood, and how drought severity interacts with the dynamics of recovery remains an open question. In this study, we performed a detailed temporal profiling of the rhizosphere and endosphere microbiomes of rice plants exposed to different drought durations. We found that the magnitude of compositional changes undergone during drought and the capacity to fully recover were significantly affected by the duration of drought stress. In particular, prolonged drought resulted in lasting changes, especially in the endosphere. Among drought-responsive microorganisms, several endospheric Actinobacteria were significantly enriched during drought and for weeks after rewatering. Notably, a highly occurring Streptomyces was identified as the most abundant member of endosphere communities during drought – constituting up to one-fourth of the entire root microbiota – and persisting into the early stages of recovery. By isolating this strain via culture, we showed that root colonization by this taxon resulted in robust root growth promotion. Collectively, these results reveal that severe drought results in enduring impacts on root-associated microbiomes that could potentially reshape the recovery response of rice plants.

HOST AND ENVIRONMENTAL DRIVERS OF PHYLLOSPHERE COMPOSITION ACROSS EUROPE

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Microbes affect plant health, stress tolerance and life history. In different host populations, plants are colonized by distinct pathogenic and commensal microbiomes, but the factors driving intra and inter-geographic variation are largely unknown. I will present two studies that explore the abiotic and biotic factors that influence the composition of the phyllosphere of Arabidopsis thaliana populations, and specifically the abundance of the bacterial pathogen Pseudomonas syringae. In the first study, we characterize the distribution and evolution of Pseudomonas pathogens across A. thaliana populations in Southern Germany. In the second study, we measured the core leaf microbiome of A. thaliana in its native range, from almost 300 populations across Europe. In these studies we discovered marked, geography-dependent differences in microbiome composition within A. thaliana and between A. thaliana and other Brassicaceae. We find evidence that host plant genetics acts to maintain different strains of *Pseudomonas* within populations as well as is associated with microbiome compositional differences across Europe. We further find that microbiome composition is best predicted by drought-associated metrics that are well known to be a major selective agent on A. thaliana populations. The reproducible and predictable associations between specific microbes and water availability raise the possibility that drought not only directly shapes genetic variation in A. *thaliana*, but does so also indirectly through its effects on the leaf microbiome.

SORGHUM ROOT MICROBIOME DYNAMICS UNDER NUTRIENT-LIMITED CONDITIONS

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Sorghum bicolor is a genetically diverse crop cultivated for a variety of agronomic uses, including grain, sugar, and energy production. However, cultivation of energy sorghum for biofuel production will require the use of marginal lands with potentially low nutrient availability and/or periods of water stress. To explore possible microbial solutions to increase the nutrient use efficiency and resilience to water stress in sorghum, we used 16S rRNA sequencing, metagenomics, and SPIEC-EASI network analysis to survey the diversity, structure, and functional potential of sorghum bacterial communities. We report how drought, nitrogen deficiency, and plant genotype alter the sorghum microbiome throughout the growing season and correlate these changes with sorghum biomass.

The sorghum rhizosphere microbiome varied considerably over the course of the growing season, with a significant drop in diversity mid-season associated with the dominance of a few selected members. Growing condition (N and water availability) had a significant effect on the alpha and beta diversity of the community, which were also influenced by sorghum genotype and location within the field. Network analysis suggested that growth condition influenced not only the abundance but also the structure and co-associations of specific beneficial bacterial taxa, with greater connectivity observed under stress. We found that sorghum dry weight and height were positively correlated with the relative abundance of putatively plant growth promoting bacteria (PGPB), regardless of genotype or growth condition, though the composition of PGPB varied by treatment. Shotgun metagenome data revealed increases over time in the abundance of bacterial gene families associated with nitrogen fixation, siderophore biosynthesis, and plant hormone biosynthesis in rhizosphere communities of abiotic stressed plants. Overall, our results suggest that under abiotic stress, sorghum individuals are able to recruit beneficial bacteria to their rhizosphere and that microbe-mediated interactions play a critical role in plant resilience.

PLANTS SPEAK CHEMISTRY: STEPS TO UNVEIL THE HIDDEN LEXICON OF CROP HOLOBIONTS FOR SUSTAINABLE AGRICULTURE

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There is an increasing interest and intense activities in the area of plant science towards harnessing the potential of plant microbiome for sustainable agricultural solutions. These new trends open a major opportunity to drive paradigm shifts towards disruptive innovation in agriculture. Public and private organizations are heavily investing in this new, fast evolving field. Among the different pursued approaches, here we present one centred on understanding the chemical signals that regulate the interactions in crop holobionts. The underlying mechanisms influencing the assembly of the microbiome remain still elusive. Using an integrated approach combining NGS, metabolomics, phenomics and organic chemistry we try to unveil the hidden lexicon regulating the microbial assembly in major crops like corn and soya. We will present how diversity in chemical signals can impact critical steps in the establishment of important symbiosis relationship such as with arbuscular mycorrhizal fungi. We will illustrate some methodological advances to gain a better temporal and spatial definition of chemical signals in the rhizosphere of important crops, especially in the perspective of developing applied solutions. We will then highlight the importance of translating the understanding of the underlying mechanisms regulating the crop holobiont to crop performances. Finally, we will discuss a roadmap relying on both fundamental and applied research and the critical role of public-private collaborations to identify new innovative and sustainable solutions for the future agriculture.

DECONSTRUCTING RHIZOSPHERE DYNAMICS USING FABRICATED ECOSYSTEMS

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Microbiomes are important drivers of plant health. Small molecule metabolites are thought to mediate many of these interactions. However, the large variability and lack of control in natural environments makes it challenging to measure the functions of exogenous metabolites in mediating microbial interactions. To address this need, we recently developed methods for making and using fabricated, single plant-scale ecosystems (EcoFABs). EcoFABs also have the potential to help standardize rhizosphere experiments, enabling researchers to build on each other's work. To advance both goals, we performed a multi-lab intercomparison study of *Brachypodium distachyon* exudates and other plant phenotypes, confirming device suitability and reproducibility across labs. We have now developed a defined rhizosphere microbiome and are organizing a second intercomparison experiment. To expand our metabolomic analyses beyond those that can be identified using authentic standards we have developed integrated metabolomics and cheminformatics approaches for exploring the chemistry of microbiomes. Using these capabilities, we now have multiple lines of evidence supporting the role of aromatic acids exudates in structuring rhizosphere microbiomes. We are now using diverse *Brachypodium distachyon* lines to further test this interaction and developing an automation platform for accelerating and expanding these rhizosphere microbiome studies.

THE FUNCTION AND GENOMIC RESOURCE OF ROOT MICROBIOME REVEALED BY FIELD-GROWN CROPS AND CULTIVATED BACTERIA

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Roots of crop plants associated with taxonomically diverse microbiota. Understanding the functions of root microbiota and systematically obtaining the cultured root microbes are crucial steps for harnessing crop microbiome in sustainable agriculture. Recently, we identified root bacterial microbiota members significantly associated with crop growth by performing large scale associations of root microbiota, growth traits and genotypic variations of field-grown crops. Using individual bacteria and synthetic community approaches, we validated the functions of root microbiota members in controlled conditions. In addition, genomes of root-derived bacterial culture collections showed the metabolic and functional capacity of root microbiota. These data and resources allow us to reveal the functions and co-adaptation of root microbiota and field-grown crops.

CYTOKININ DRIVES ASSEMBLY OF THE PHYLLOSPHERE MICROBIOME THROUGH STRUCTURAL AND CHEMICAL CUES

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Cytokinin (CK) is an important developmental regulator, promoting morphogenesis and delaying differentiation and senescence. CKs are also known to mediate plant immunity. Several classes of microbes can also produce CKs, affecting the interaction with their plant hosts. While host species and genotype can be a driving force in shaping the plant microbiome, how CK can shape the microbiome, as well as the relationships between plant developmental status and microbiome assembly, are largely uninvestigated. Here, we examined the relationship between CK and the phyllosphere microbiome, finding that CK acts as a selective force in microbiome assembly, increasing richness, and promoting the presence of Firmicutes. Using a biomimetic system, we investigated the relationship between the leaf microstructure, and the growth of different phyllosphere microbes. We observed that leaf structures derived from CK-rich plant genotypes support bacilli in the biomimetic system. Exploring the relationships between plant development and microbiome assembly, we found that age-related shifts in microbiome content vary based on content of, or sensitivity to, CK. We observed a developmental age associated decline in microbial richness and diversity, accompanied by a decline in the presence of growth promoting and resistance inducing bacilli in the phyllosphere. Bacillus isolates we obtained from CK rich genotypes were found to re-program the transcriptome to alter the leaf developmental program in seedlings, and enhance agricultural productivity in mature plants. Overall, our results indicate that genotype and hormonal profiles can act as a strong selective force in microbiome assembly, and that CK-dependent effects on microbiome content support developmental functions.

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PRECURSOR-DIRECTED ACTIVATION OF MICROBIAL VOLATILES TO SUPPRESS SEED GERMINATION OF THE PLANT PARASITIC WEED STRIGA HERMONTHICA

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Striga hermonthica is a root parasitic weed responsible for substantial staple crop losses across sub-Saharan Africa. Here we investigated the functional potential of the soil microbiome to interfere with the initial stages of the parasite's life-cycle. More specifically, we investigated the impact of microbial volatile production on strigolactone-induced Striga seed germination. Using a two-compartment Petri dish, we screened approximately 200 bacterial strains, representing taxonomically diverse families, for volatile-mediated suppression of *Striga* seed germination. More than 50% of the strains showed some degree of volatile-mediated suppression of *Striga* seed germination with the majority (79%) belonging to the genera Janthinobacterium and Collimonas. Comparative GC-profiling of the volatiles emitted by 55 strains pinpointed the sulfurous volatile compounds dimethyl disulfide (DMDS) and dimethyltrisulfide (DMTS) as candidate inhibitors of *Striga* germination, which was validated in dose-response activity assays with the pure compounds. Since application of these volatiles to the soil matrix can be cost prohibitive for smallholder farmers, we tested if methionine, the precursor of these sulfurous volatiles, stimulated their production in soils and led to a concomitant suppression of Striga seed germination. Results showed that addition of L-methionine to soil indeed led to the production of the two Striga-suppressive sulfurous volatiles and a significant suppression of Striga seed germination in a concentration-dependent manner. Together, these results revealed the functional potential of soil microorganisms to interfere in the *Striga* life-cycle and the use of specific precursors to activate the indigenous soil microbiota for the production of specific volatile compounds to effectively combat this devastating parasitic weed.

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SEASONALITY AND SHELF LIFE ARE MAIN DRIVERS OF THE MICROBIOME AND E. COLIO157:H7 SURVIVAL ON COLD-STORED LETTUCE CULTIVATED IN A MAJOR PRODUCTION AREA IN CALIFORNIA

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Lettuce is linked to recurrent outbreaks of Shiga toxin-producing E. coli (STEC) infections, the seasonality of which remains unsolved. Infections have occurred largely from processed lettuce, which undergoes substantial physiological changes during storage. We investigated the effect of shelf life on the microbiome and STEC O157:H7 (EcO157) colonization of fresh-cut romaine lettuce of two cultivars harvested in the spring and fall in California and stored in modified atmosphere packaging (MAP) at 6°C. Inoculated EcO157 declined significantly less during storage on the cultivar with short shelf life. Metagenomic sequencing of the lettuce microbiome revealed that the pre-storage bacterial community was variable but dominated by species in the Erwiniaceae and Pseudomonadaceae. After cold storage, the microbiome composition differed between cultivars, with a greater relative abundance (RA) of Erwiniaceae and Yersiniaceae on the cultivar with short shelf life. Fall harvest followed by lettuce deterioration were identified by recursive partitioning as important factors associated with high EcO157 survival. Both pre- and post-storage microbiomes differed by season. High representation of Pantoea agglomerans was a predictor of fall microbiomes, lettuce deterioration, and enhanced EcO157 survival. In contrast, higher RAs of Erwinia persicina, Rahnella aquatilis, and Serratia liquefaciens were biomarkers of spring microbiomes and lower EcO157 survival. Our results strongly support a role for season and lettuce deterioration in EcO157 survival and microbiome composition, suggesting that the physiology and microbiomes of fall- and spring-harvested lettuce may contribute to the seasonality of STEC outbreaks associated with lettuce grown in coastal California.

HYBRIDIZATION, HETEROSIS, AND THE MAIZE MICROBIOME

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Hybrids account for nearly all commercially planted varieties of maize and many other crop plants, because crosses between inbred lines of these species produce F_1 offspring that greatly outperform their parents. The mechanisms underlying this phenomenon, called *heterosis* or hybrid vigor, are not well understood despite over a century of intensive research. The leading hypotheses—which focus on quantitative genetic mechanisms (dominance, overdominance, and epistasis) and molecular mechanisms (gene dosage and transcriptional regulation)-have been able to explain some but not all of the observed patterns of heterosis. Abiotic stressors are known to impact the expression of heterosis; however, the potential role of microbes in heterosis has largely been ignored. Here we show that heterosis of root biomass and other traits in maize is strongly dependent on the belowground microbial environment. We found that, in some cases, inbred lines perform as well by these criteria as their F_1 offspring under sterile conditions, but that heterosis can be restored by inoculation with a simple community of seven bacterial strains. We observed the same pattern for seedlings inoculated with autoclaved vs. live soil slurries in a growth chamber, and for plants grown in steamed or fumigated vs. untreated soil in the field. In a different field site, however, soil steaming increased rather than decreased heterosis, indicating that the direction of the effect depends on community composition, environment, or both. Deep sequencing of the 16S rRNA gene and internal transcribed spacer confirmed that field-grown hybrid and inbred plants host distinct bacterial and fungal microbiomes in the rhizosphere, and that seven out of seven hybrids tested showed heterosis for microbiome composition. Together, our results demonstrate a novel, ecological phenomenon whereby soil microbes differentially impact the early growth of inbred and hybrid maize.

POSTER ABSTRACTS

(IN ALPHABETICAL ORDER BY LAST NAME)

DECIPHERING THE SORGHUM RHIZOSPHERE MICROBIOME TO IDENTIFY BACTERIAL SPECIES AND CONSORTIA FOR *STRIGA* CONTROL

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Sorghum is a major staple crop in Sub-Saharan Africa and Asia and essential for food security. However, its production is severely hampered by the plant parasitic 'witch weed' Striga hermontica. Despite significant advances in resistance breeding, chemical intervention and cultural practices, the *Striga* problem is not resolved yet. Here, we are exploring the functional potential of the root microbiome as a novel and complementary strategy to disrupt the Striga lifecycle and to enhance sorghum yields. To this end, we investigated the rhizosphere microbiome composition of 12 sorghum genotypes, including wild relatives, landraces and improved sorghum varieties. Out of 48 agricultural field soils collected from the center of origin of sorghum, Ethiopia, the 12 genotypes were grown in three microbiologically and physicochemically distinct soils. This approach maximizes bulk soil microbiome diversity and minimizes the core community overlap. The results showed that the core microbiome of sorghum comprised a total of 2,125 ASV's belonging to eight bacterial families. Despite the genotypic and soil type effects on sorghum rhizosphere microbiome composition, we observed the persistent presence of differentially abundant and indicator species correlated with specific sorghum genotypic traits. We also provide a link between the sorghum rhizosphere microbiome diversity and plant genotypic distance, by correlating the sorghum DArT-SNP profile and the corresponding microbiome distance matrix. This study sets the baseline to use the core and accessory sorghum rhizosphere microbiome for identifying and applying bacterial species and consortia to further control Striga in sorghum.

INTEGRATION OF GENOMIC AND QUANTITATIVE STRAIN-LEVEL METAGENOMIC PROFILES BY USING qRRS IN THE SWEETPOTATO PHYLLOSPHERE

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Functional and quantitative metagenomic profiling remains challenging and limits our understanding of plant-microbiome interactions. We present a novel quantitative reduced representation sequencing (qRRS) strategy, which leverages the strengths of shotgun and amplicon sequencing. We characterized the sweetpotato leaf microbiome in two biparental populations (total of 764 F1 progenies) and one diversity sweetpotato panel (767 accessions). The metagenome profiles and high-density SNP data were integrated to identify host genetic factors that underpin host-microbiome interactions. Using the metagenome profile as a covariate for GWAS (metagenomic-enhanced GWAS) revealed increased statistical power for 49.4% of plant-microbe interactions (2.4% lost statistical power and 48.2% remained unchanged). Correlation network analysis revealed that strain/species-level profiles were more sensitive at detecting microbe-microbe interactions and microbial hubs. These signals rapidly dissipated at higher taxonomic levels. Potential biocontrols were found to be enriched within the microbiome (relative abundance) and are likely key modulators of the microbial community, possibly through quorum sensing. The metagenomic profile prediction of population structure, particularly in the bi-parental populations, agreed with SNP data-derived population structure. The F1 progenies from the biparental population mostly recruit the same microbes recruited by their parents, thus demonstrating the strong impact of host genetics on microbiome recruitment. This study highlights a low-cost, quantitative and strain/species-level metagenomic profiling approach; new tools that complement the assay's novel features and provide fast computation; and the potential for advancing functional metagenomic studies.

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BACTERIAL ENDOPHYTES CONTRIBUTE TO RICE SEEDLING ESTABLISHMENT UNDER SUBMERGENCE

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Rice can germinate and grow successfully under oxygen shortage. Previous studies showed that rice plants host a wide range of endophytic bacteria, capable of producing plant growth promoters that support plant development and survival under abiotic stress conditions. Currently, the role of endophytic bacteria under hypoxia is still poorly defined. We studied *japonica* rice varieties, showing contrasting phenotypes when germinating under submergence, for bacterial endophytes composition, using metagenomics, *in vitro* isolation and PGP assays to uncover their possible role under hypoxia. Our findings suggest that endophytic bacteria can contribute to rice seedling establishment under submergence and that diverse rice genotypes may benefit differently from bacteria inocula.

ANTAGONISTIC ACTIVITY AGAINST VERTICILLIUM DAHLIAE OF ENDOPHYTIC BACTERIAL STRAINS ISOLATED FROM THE XYLEM OF OLIVE TREES

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Olive is one of the most important cultivated trees in the Mediterranean Basin and exhibits a wide range of noteworthy environmental attributes. Nowadays, however, its health status is seriously threatened by phytopathogenic organisms such as the fungus Verticillium dahliae and the bacterium *Xylella fastidiosa* that obstruct xylem sap flow leading to wilting of plant tissues. Biological control agents represent a promising strategy to control these plant pathogens. We investigated the potential of xylem-inhabiting microorganisms that may compete for infection sites, antagonize the pathogen, and/or induce host plant defense responses, thus contributing to mitigate disease development. A total of 108 bacterial strains isolated from xylem vessels of different cultivated and wild olive genotypes were used to test their antagonistic activity against three Verticillium dahliae strains isolated from olive (defoliating and non-defoliating strains) and tomato. Antagonistic activity was tested in olive xylem sap and in three predefined culture media with chemical composition similar to that of olive xylem sap, using a high-throughput fungalbacterial interaction screen method. Results based on a Relative Growth Index indicated that 29 strains restrict V. dahliae (RGI<-1.2) and 30 strains promote fungal growth (RGI>0.2), while 27 strains are neutral regarding V. dahliae (-0.05<RGI<0.05). Overall, specific Methylobacterium, Microbacterium and Rhizobium strains restricted fungal growth, whereas other Methylobacterium, Bosea and Sphingomonas strains promoted V. dahliae fungal growth.

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RECRUITMENT OF THE RHIZOSPHERE MICROBIOME IN RESPONSE TO DROUGHT

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Global climate change has resulted in losses to agricultural productivity. Mitigating the effects of climate-related stress on crops is important for global food security and crop improvement. The belowground microbial community associated with plant roots, henceforth, the rhizobiome, can recruit beneficial microbes to alleviate effects of stress. However, the factors influencing the recruitment of the rhizobiome during stress are not completely understood. We conducted a greenhouse experiment to investigate the responses of rhizobiome bacterial communities from two crop species- switchgrass (Panicum virgatum) and common bean (*Phaseolus vulgaris*). We collected field rhizosphere soils from each crop, divided them into planted and unplanted treatments, and exposed them to a gradient of drought severity over time. With this experiment, we aimed to understand both general and specific rhizobiome member responses to drought across the two plant species, the impact of drought severity on rhizobiome recruitment, and the consequences of direct drought effects versus plant mediated effects on the rhizobiome. 16S-V4 rRNA gene amplicon sequencing revealed differences in community structure between the rhizobiome of bean and switchgrass (PERMANOVA P=0.001). Within each crop, there were differences in community structure between planted and unplanted soil samples (PERMANOVA P=0.001), suggesting plantmediated responses. Because plants release hormones such as abscisic acid and salicylic acid during drought, we also performed in-vitro experiments to determine the responses of rhizobacteria, bulk soil bacteria and pathogens to a gradient of these phytohormones. Together, these results contribute to the development of microbiome management strategies during drought by helping to identify responsive, beneficial taxa.

PLANT MICROBIOME ADAPTATION TO HARSH ENVIRONMENTS

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Recent research indicates that every plant is endowed with its own adaptable microbiome. A growing body of literature shows that plant health, growth, and biomass production are influenced by microbiomes. Interactions between host plant and microbiome have been successfully exploited by application of selected bacteria or fungal strains onto crops, e.g. to enhance plant growth and stress resistance in wheat and increase yields of nutrient-stressed barley cultivars. This suggests great potential for engineering beneficial microbial communities.

Recently dramatic within-species population-level differences in resilience to extreme saline and serpentine environments in several tractable *Brassicaceae* species have been observed. A subset of *Arabidopsis arenosa* populations grown in drought prone serpentine sites, while a subset of *Brassica fruticulosa* populations are locally adapted to high salt conditions, whereas other populations in both species are not adapted to these challenges. As for many plant species beneficial roles in abiotic stress adaptation mediated by the microbiome have been described, we hypothesise that wild plant populations may accumulate beneficial microbes to withstand environmental stresses. Therefore, we are interested in the plant microbiome communities of the wild *Brassicaceae* ecotypes. For this we present bacterial and fungal microbiome atlases studying *Brassica fruticulosa* populations adapted to high saline soils and *Arabidopsis arenosa* populations adapted to serpentine soils – both relevant stressors for worldwide agricultural challenges. We show that the selected ecotypes enrich different microbiomes under abiotic stress conditions and detect amplicon sequence variants of potentially interesting microbial candidates involved in enhancing saline and serpentine adaptation.

PHYLLOSPHERE RESIDENTS VERSUS XANTHOMONAS SP.: A FOLIAR PATHOGEN'S INFLUENCE ON THE PHYLLOSPHERE MICROBIOME

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The importance of microbiome components in mediating the plant-pathogen interactions has gained a particular attention in the recent years. The goal of this study is to dissect interactions of the pathogen with the host microbiome to assess their influence on overall host susceptibility. In this study, the phyllosphere of tomato plants were surveyed through culture-dependent competition assays of isolated bacterial residents against foliar pathogen, Xanthomonas *perforans*. A library of 128 phyllosphere residents, composed of approximately 21 genera, was constructed from tomato fields throughout the Southeastern U.S., and identified through 16S sequencing. A total of 17 phyllosphere isolates inhibited the foliar pathogen, X. perforans and contrarily 3 phyllosphere isolates were shown to be inhibited by X. perforans. We further dissected these positive and negative interactions to understand the mechanistic basis. Five isolates showed involvement of bacteriocins in inhibition of *Xanthomonas*. Prior research from our lab indicates contribution of TssM, a core component of Type VI Secretion System (T6SS), towards initial asymptomatic colonization of the pathogen in the tomato phyllosphere. This observation and the well-established research suggesting involvement of T6SS in mediating interbacterial competition in other pathosystems led us to hypothesize that the functional TssM of the pathogen is important for creating its niche in the phyllosphere and its ability to overcome competition with resident microflora. In vitro contact-dependent assays revealed that functional TssM was important for mediating either positive or negative interactions with 9 isolates. This study sheds light on how pathogens adapt to/modify the phyllosphere microbiome during host colonization.

BURKHOLDERIACEAE AND MULTIDRUG RESISTANCE GENES ARE KEY PLAYERS IN RESISTOME DEVELOPMENT IN A GERMFREE SOIL MODEL

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Assembly of a resistome in parallel with the establishment of a microbial community is not well understood. The use of germfree models to study bacterial colonization and resistance evolution under antibiotic pressure is becoming increasingly important. In this study, we exposed germfree soil (GS), GS with diluted non-treated soil (DS), and non-treated soil (NS), to various concentrations of tetracycline (TET) in a non-germfree environment for 10 weeks, followed by 2 weeks exposure to water. High-throughput sequencing was used to profile bacterial communities and antibiotic resistance genes (ARGs) in the soils. The initial bacterial loads were found to shape the profiles of bacterial communities and the resistomes. GS and DS treated with or without TET had similar profiles, whereas NS showed different profiles. Soils with the same initial bacterial loads had their profiles shifted by TET treatment. Multidrug resistance (MDR) genes were the most abundant ARG types in all soils, with the multidrug efflux pumps being the discriminatory ARGs in GS regardless of different TET treatments, and in GS, DS, and NS after TET. Furthermore, MDR genes were significantly enriched by TET treatment. On the contrary, tetracycline resistance genes were either absent or low in relative abundance. The family Burkholderiaceae was predominant in all soils (except in NS treated with water), and was positively selected for by TET treatment. Most importantly, Burkholderiaceae was the primary carrier of ARGs, including MDR genes.

OLIVE-ASSOCIATED FUNGI AS A NEW SUSTAINABLE APPROACH TO OLIVE CROP PROTECTION AGAINST ANTHRACNOSE

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The olive tree (Olea europaea L.) plays an important socio-economic role worldwide, but olives are frequently affected by anthracnose caused by *Colletotrichum* spp.. This disease is considered one of the most destructive around the world and leads to premature fall and/or mummification of fruits, with a consequent reduction in production and/or depreciation of olive oil quality. The susceptibility of olives to anthracnose is already known to be affected by different factors, such as cultivar, production system and olive maturation stage. The control of this disease is very difficult and mostly relies on the use of copper based fungicides. The current awareness of the impact of these products on health and environment has highlighted the importance of creating ecological alternatives, such as biocontrol approaches. The plant-associated microbial community has been increasingly recognized as playing an important role in plant health. The main objective of this work was to evaluate the endophytic and epiphytic fungal communities of olives to explore the potential of native olive microbiota as biological control agents against anthracnose. Using a metabarcoding approach, through sequencing (Illumina MiSeq) of ITS amplicons, fungal diversity was determined in olives from different cultivars (Cobrançosa and *Madural*), production systems (organic farming and integrated production) and in two maturation stages (green and semi-ripen). The results allow a better understanding of the effect of the cultivar, the production system of the olives and maturation stage of the fruit in the structuring of the fungal community. Results are discussed concerning the selection of potential biocontrol agents against anthracnose.

MICROBIOME ANALYSIS OF CARYAILLINOINENSIS SEEDLINGS

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Carya illinoinensis (pecan) possess genetic adaptations to grow in a broad environmental spectrum as its native region extends from Illinois, USA to Oaxaca, MX. Commercial cultivation has expanded throughout the United States and worldwide. Recent studies have shown that the plant microbiome may be influenced by both the environmental conditions and genetics of the plant. We sought to analyze the microbiome of pecan seedlings and determine if microbial composition differed among organs. Seeds were collected from five maternal pecan genotypes. The seeds were stratified, planted in a soilless potting mix, and grown in a quarantine greenhhouse. Roots, stems, and leaves were collected for DNA extraction, and next generation sequencing was performed. Quality control, clustering, and statistical analysis were performed with CLC-Genomics Workbench 21.0.4 using the Microbial Genomics Module. For operational taxonomic unit (OTU) clustering and identification, the SILVA 132 at 97% bacterial database and Unite 99% fungal database was used as references. Results indicated a significant difference in microbial composition with roots having the highest alpha diversity followed by stems and leaves. Differences in microbial composition and abundances were also observed between the tissues. Microbiome research is in its early stages in pecan and elucidating pecan microbiomes within different genetics backgrounds and geographical regions is important to determine the role the microbiome is playing in the tree's ability to adapt to a wide geographical range. In the future, manipulation of the microbiome will be possible to optimize the cultivation of pecan trees in specific environmental conditions.

MICROWELL RECOVERY ARRAY SCREENING OF MAIZE ROOT-ASSOCIATED BACTERIA TO IMPROVE AZOSPIRILLUM BRASILENSE COLONIZATION AND PLANT GROWTH

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Plant growth-promoting bacteria (PGPB) are key for sustainable agriculture and may alleviate the negative impacts of chemical fertilizers on human health and the environment. Successful application of PGPB requires colonization of the root microbiome, which is influenced by interactions with other microbes. Using a novel microwell recovery array (MRA), bacteria that improve Azospirillum brasilense colonization in maize (Zea mays L.) roots can be rapidly discovered and isolated. In a single test, the MRA recovered several isolates that promote the growth of A. brasilense, both within the MRA and in off-chip validation assays. The identified isolates were then co-inoculated with A. brasilense on axenic maize seedlings inside a plant growth chamber. After 15 days of growth, co-inoculated plants grew taller than plants inoculated with only A. brasilense and A. brasilense colonized roots better when MRA promoter isolates were present. To test whether the isolates increased A. brasilense growth promotion in a more realistic plant growth system, a second growth chamber experiment was conducted in which maize was grown in calcined clay. After 30 days of growth, A. brasilense altered root system architecture relative to the sterile control with one isolate amplifying that effect. A. brasilense coinoculation with several isolates also resulted in increased shoot and root biomass relative to the A. brasilense monoculture. These findings uncover new interactions useful for developing improved PGPB consortia and demonstrate that the MRA tool can rapidly explore complex environmental microbiomes to discover new isolates capable of generating positive host phenotypes.

THE IMPACT OF DIFFERENT CLIMATE CONDITIONS AND DISEASE SEVERITY LEVELS ON CORK OAK FUNGAL ENDOPHYTIC COMMUNITY

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Cork oak is an evergreen tree species widely distributed in the Mediterranean region, in which has high ecological and socio-economic importance. This region is described as a climate change hotspot, in which predicted effect of decreased precipitation and increase of warming will endanger cork oak forests sustainability and productivity. Cork oak decline has increased in the last decades and climate changes pose greater challenges to this tree species. Extreme events (e.g., prolonged drought) are described to weaken trees and change plant-associated microbial communities, which is advantageous for colonization of opportunistic pathogens. Several questions were raised: i) Are cork oak microbial changes a response to different environmental conditions?; ii) Are tree disease severity levels correlated with environmental conditions and/or to shifts on microbial communities?; and iii) How these changes can potentiate colonization by opportunistic pathogens? To answer these questions, twigs and leaves from cork oak trees, located on forests under different environmental conditions (e.g., bioclimate) and different disease severity levels were collected. Plant tissues were surface sterilized, and DNA was extracted to obtain only endophytic communities. Fungal community was sequenced using ITS2 barcode region with Illumina MiSeq technology. Endophytic communities from different bioclimates displayed differences at family and genus level. Several opportunistic pathogens were identified, in which higher incidence was displayed on driest forests. These results contribute to understand the consequences of climate changes on cork oak health and create an opportunity to apply endophytic communities for better management and sustainability of cork oak forests.

A PROTEO-GENOMICS DISSECTION OF THE WALNUT-XANTHOMONAS ARBORICOLA PV JUGLANDIS INTERACTOME

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Walnut blight (WB), caused by Xanthomonas arboricola pv. juglandis (Xaj), significantly decreases walnut production worldwide. We focused on a copper-resistant isolate (Xaj417) to define the proteo-genomics components that mediate host-pathogen interaction leading to disease. Our results revealed that orchard management practices select for pathogenic strains. In addition, bacterial virulence and copper resistance emerged by the acquisition of sets of pathogenesis-related genes commonly transferred among Xanthomonas on mobile genetic elements (MGE). This was evidenced for Xaj417 genome, with the presence of a horizontally acquired copper resistance cassette. The expansion of MGE among virulent strains influences the growing repertoire of virulence effectors and adaptation strategies shaping the evolution of pathogenic strains. We dissected this pathosystem using TMT quantitative proteomics demonstrating an increased abundance of type II secretion effectors during WB including a secreted chorismate mutase (CM). The dysfunctional mutant (XajCM) showed increased virulence in walnut. Xaj proteins detected in infected hull tissues demonstrated their ability to adapt to the host microenvironment, limiting iron availability, coping with copper toxicity, while maintaining energy and intermediary metabolism. Interestingly, the most abundant protein in XajCM was a polygalacturonase while in the host tissue, it was a polygalacturonase inhibitor. Thus, CM may be an important virulence suppressor gene that regulates virulence allowing improved bacterial survival within the plant tissues. Overall, this study offers new insights into the emergence of virulence, adaptation, and tolerance to disease management strategies used in orchard ecosystems and provides insight into disease mechanisms, potential molecular tools for early detection, and disease limiting strategies.

HERITABILITY AND HOST GENOMIC DETERMINANTS OF SWITCHGRASS ROOT-ASSOCIATED MICROBIOTA

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A fundamental goal in plant microbiome research is parsing the effects of host vs. environment when determining the composition of root microbiota, particularly how host genotypes impact community composition. Most studies characterizing the "genotype effect" on root microbiota undersample host genetic diversity and grow plants outside of their native ranges, making the associations between host and microbes difficult to interpret. Here we characterized root microbiota of a large population of switchgrass, a North American native C4 bioenergy crop, in three field locations spanning its native range. Our data, composed of >2000 samples, suggest field location is the primary determinant of microbiome composition, but significant heritable variation is widespread for individual microbes. Bacteria within Sphingomonadaceae displayed elevated heritability estimates compared to other clades. Using a genome wide association study (GWAS) framework, we identified loci impacting the abundance of >400 microbial strains, finding an enrichment of genes involved in immune responses, signaling pathways, and secondary metabolism. We found loci associated over half of the core microbiota (i.e. microbes in >90% of samples) regardless of field location. Finally, we developed a collection of > 300unique isolated bacterial strains and showed that core microbiota have strong effects on switchgrass seedling growth. One core Sphingobium strain decreased root length, but increased root hair density. GWAS for this strain revealed a locus in linkage with the transcription factor ROOTHAIRLESS1 ortholog from Lotus, implicating the role of root hairs in microbial colonization. This study will bring us closer to harnessing and manipulating beneficial microbial associations via host genetics.

THE EFFECTS OF PLANT IMMUNITY ON MICROBIOTA DEPEND ON HABITAT AND MICROBIAL KINGDOM

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The plant immune system recognizes, responds to, and limits the growth of invading microorganisms, yet the plant microbiome contains multitudes of microbes. The extent to which host immunity actively discriminates between commensal and pathogenic microorganisms or commensals bypass immunity is unknown. Using Arabidopsis mutants we targeted broad sectors of plant immunity - MAMP recognition, MAMP signaling, and hormone-related immune outputs - to understand how perturbing immune function alters microbiome assembly. We enumerated the bacterial and fungal communities across epi- and endophytic habitats in the leaf and root organs of wild soil grown plants. The sectors of immunity that we ablated had little effect on the abundance of bacteria or fungi in any habitat with the exception of the MAMP signaling mutant, which had elevated bacterial load in the habitat tightly attached to the root surface (rhizoplane). Compositional effects of plant immunity could be large but depended on plant habitat, where effects were strongest in root endophytic and rhizoplane bacterial communities. We observed a striking modular pattern of differential abundance where MAMP recognition and MAMP signaling mutants enriched one set of bacterial taxa in the rhizoplane and depleted another set in the root endophytic compartment. In contrast, the hormone mutant exhibited a general enrichment of both of these modules. The perturbed taxa were taxonomically diverse and accounted for 36% and 17% of the total rhizoplane and root endophytic microbiome of Col-0, respectively. These results highlight the contrasting effects of different sectors of immunity on two kingdoms of microbiota across shoot and root habitats.

CROWN CLOSURE AFFECTS ENDOPHYTIC LEAF MYCOBIOME COMPOSITIONAL DYNAMICS OVER TIME IN PSEUDOTSUGA MENZIESII VAR. MENZIESII

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Old-growth coastal Douglas-fir forests produce complex environmental and spatial gradients along which biota assemble. It has been proposed that changes in the crown microenvironment are associated with different community assembly outcomes for needle fungi. With high-throughput sequencing, the endophytic mycobiomes of needles were characterized for increasing ages of needles sampled along the boles of eight P. menziesii trees. Leveraging airborne laser scanning data to describe tree crowns revealed that crown closure and depth in crown accounted for more compositional variation than height in crown, and richness was correlated with crown closure. Supplementing point clouds from climbed trees with clouds from >5,000 randomly selected trees in the study area showed that needle communities from closed portions of the crown were more diverse and structured with increasing needle age. These findings highlight the importance of the microenvironment relative to the development of canopy myconsortia for a foundation tree species.

INFLUENCE OF PLANT SECONDARY METABOLITES ON FUNCTIONAL TRAITS OF THE POLYCHLORINATED BIPHENYL (PCB)-DEGRADING STRAIN PARABURKHOLDERIA XENOVORANS LB400

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Plant secondary metabolites play an important role in building relationships with the plantassociated microbiota, essential for maintaining the health status of the holobiont under stress and its services. Efficient interactions between plants and beneficial bacteria are fundamental in rhizoremediation strategies in order to degrade contaminants present in the soil or increase their bioavailability. Exploiting these relationships is particularly useful for the biodegradation of highly recalcitrant and phytotoxic contaminants such as polychlorinated biphenyls (PCBs). Some root-exudated compounds, mostly secondary metabolites like flavonoids, showed to efficiently biostimulate the catabolic metabolism of PCB-degrading bacteria. Nevertheless, the mechanisms underlying this dialogue and the role played by root exudates are still poorly understood.

The involvement of flavonoids in the stimulation of metabolism and on functional traits related to rhizocompetence was investigated in the PCB-degrading bacterium *Paraburkholderia xenovorans* LB400 by *in vitro* assays. Increasing concentrations of the flavonoids naringin, naringenin and quercetin led to a stimulation of bacterial growth in terms of biomass accumulation. Naringin also showed to have a positive influence on bacterial swimming motility. *In planta* experiments on *Arabidopsis thaliana* mutants with an altered root exudation pattern exposed or not to PCB stress were also carried out to compare colonization efficiency by the bacterial strain.

The results obtained contribute to understand the functioning of the interactions between plant and bacteria. In addition, they provide useful information to increase rhizoremediation efficiency in PCB-contaminated soils, exploiting metabolites naturally present in the root system of the plant.

DROUGHT ADAPTED SOIL MICROBIAL COMMUNITIES INCREASE PLANT DROUGHT TOLERANCE

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Soil microbes encounter free-living and plant-associated environments, meaning they have the potential to evolve independently or closely intertwined with a host. It is unclear whether, or how, a plant affects the evolution of soil microbiomes, or how soil microbes' evolutionary histories shape their effects on host phenotypes. We explore these questions in the context of plant and microbial adaptation to a model stress: water limitation. We performed a mesocosm experiment with pristine prairie soils collected from six sites along a precipitation gradient. Soil mesocosms were evolved for 5 months in one of four treatments: a factorial combination of +/water-stress and +/- plant host. Soil microbiomes subjected to a host environment were planted with Tripsacum dactyloides (gamagrass), a native prairie grass and relative of maize. After 5 months, experimentally evolved rhizosphere/soil microbiomes were inoculated onto maize or gamagrass under water-stressed conditions. Plant phenotypes were measured to understand the functional consequences of each evolved microbiome. Preliminary results indicate that soil microbiomes originating from dry environments increase water-stressed gamagrass root and shoot mass. Additionally, communities experimentally evolved under water-stressed conditions increased water use efficiency and shoot growth of gamagrass, but not maize. This suggests that drought-adapted soil microbial communities benefit prairie plants with which they share an evolutionary history, but may not benefit domesticated hosts. Lastly, microbiomes experimentally evolved with a host present had varying soil type-dependent effects on gamagrass/maize above-ground phenotypes. Metagenomic, transcriptomic, and extensive root physiological analyses are underway to further understand how drought- and host-mediated adaptations impact host-microbiome interactions.

THE BEAUTY OF YEASTS: DIVERSITY AND FUNCTIONS IN THE WHEAT PHYLLOSPHERE

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The plant microbiome has received enormous attention in the past decade and revealed a wealth of new knowledge on bacteria and fungi and their beneficial or deleterious effects on plant growth and health. Relatively little attention has been given to the diversity and functions of yeasts living on and in plant tissues. Their spatio-temporal dynamics, resilience to environmental stresses and the mechanisms by which they interact with other members of the plant microbiome remain largely elusive. Here we investigated the taxonomic and functional diversity of yeasts colonizing the surface and internal tissues of wheat flag leaves. Partial ITS sequencing of 176 yeast isolates revealed a diverse composition consisting of 13 different genera. These isolates were further characterized for their carbon utilization, biofilm formation and antifungal activity, important traits for survival in the phyllosphere. Overall, the yeast isolates utilized 16 out of 31 carbon sources tested. Biofilm formation was observed for members of the *Metschnikowia*, Vishniacozyma and Aureobasidium genera. A total of 27% of the yeast isolates inhibited the growth of the fungal foliar pathogens Fusarium graminearum and Zymoseptoria tritici via diffusible compounds. Additionally, a number of isolates also showed antifungal activity via volatile compounds. Synthetic communities will be designed for assessing their ability to protect plants against the fungal pathogens in vivo. To further explore the genetic potential of the phyllosphere yeasts comparative genome analysis will be performed. These findings will contribute to developing a new microbiome-based strategy to improve tolerance of wheat plants to fungal foliar infections.

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SYNCHRONIZED IDENTIFICATION OF METABOLIC PATHWAYS AND FUNCTIONAL MICROBIAL PLAYERS FOR METHANE PRODUCTION USING MULTIPLE ISOTOPE-TRACKING APPROACHES

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Methane is a greenhouse gas, but can also serve as clean energy. Its generation and release by microbial guilds have significant influence on biogeochemical carbon cycling and global climate change. Due to high complexity of the environmental microbiome, an *in-situ* quantitative analysis of methane-production pathways and the corresponding microbial players is a bottleneck for understanding the mechanism of methane cycle. In this study, four ¹³C and ¹⁵N probing approaches were applied in parallel in two anaerobic methanogenic niches with elevated ammonia concentrations, to quantitatively analyze the methanogenic pathways and simultaneously identify the functional microbial members by tracking the flow of stable isotopes into the metabolites and microbial cells. In the four experimental sets, [1,2-¹³C]-CH₃COOH, [2-¹³C]-CH₃COOH, [¹³C]-NaHCO₃ or non-labeled CH₃COOH, with or without [¹⁵N]-NH₄Cl were used individually, to realize a comparison of pathway quantification by labeled-¹³C or by nature-¹³C fractionation effects during methanogenesis. Both approaches quantitatively recorded the shift of predominant pathway from acetolastic (AM) to hydrogenotrophic methanogenesis (HM) with increasing ammonia concentration. HM occurred in couple with acetate oxidation (SAO) by a group of syntrophic bacteria. DNA-SIP with high-through-put sequencing identified the major microbial catalyzers, especially highlighted the roles of Methanoculleus for HM and Symbiobacteraceae for SAO. Fluorescence in situ hybridization (FISH) and qPCR monitored the gradual change of cell morphology and abundance of these members. Interestingly, NanoSIMS with FISH detected the synchronized autotrophic utilization of CO₂ by some archaeal and bacterial members. This work provided a multi-dimensional and fundamental view of methane generation by the environmental microbiome.

NETWORK MAPPING OF ROOT-MICROBE INTERACTIONS IN ARABIDOPSIS THALIANA

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Understanding how plants interact with their colonizing microbiota to determine plant phenotypes is a fundamental question in modern plant science. Existing approaches for genomewide association studies (GWAS) are often focused on the association analysis between host genes and the abundance of individual microbes, failing to characterize the genetic bases of microbial interactions that are thought to be important for microbiota structure, organization, and function. Here, we implement a behavioral model to quantify various patterns of microbemicrobe interactions, i.e., mutualism, antagonism, aggression, and altruism, and map host genes that modulate microbial networks constituted by these interaction types. We reanalyze a rootmicrobiome data involving 179 accessions of Arabidopsis thaliana and find that the four networks differ structurally in the pattern of bacterial-fungal interactions and microbiome complexity. We identify several fungus and bacterial hubs that play a central role in mediating microbial community assembly surrounding A. thaliana root systems. We detect 1142 significant host genetic variants throughout the plant genome and then implement Bayesian networks (BN) to reconstruct epistatic networks involving all significant SNPs, of which 91 are identified as hub QTLs. Results from gene annotation analysis suggest that most of the hub QTLs detected are in a proximity to candidate genes, executing a variety of biological functions in plant growth and development, resilience against pathogens, root development, and abiotic stress resistance. This study provides a new gateway to understand how genetic variation in host plants influence microbial communities and our results could help improve crops by harnessing soil microbes.

HOW MANY MICROBES DOES IT NEED TO ALLOW EFFICIENT ORGANIC NITROGEN RECYCLING IN ARBUSCULAR MYCORRHIZAL HYPHOSPHERE?

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Arbuscular mycorrhizal (AM) fungi lack efficient exoenzymes to access organic nutrients directly. Nevertheless, the fungi often obtain and further channel to their host plants a significant share of nitrogen (N) and/or phosphorus from such resources, presumably via cooperation with other soil microorganisms. Because it is challenging to disentangle individual microbial players and processes in complex soil, we took a synthetic approach here to study ¹⁵N-labelled chitin (an organic N source) recycling via microbial loop in AM fungal hyphosphere. To this end, we employed a compartmented in vitro cultivation system and monoxenic culture of Rhizophagus *irregularis* associated with *Cichorium intybus* roots, various soil bacteria, and the protist *Polysphondylium pallidum.* We showed that upon presence of *Paenibacillus* sp. in its hyphosphere, the AM fungus (and associated plant roots) obtained several-fold larger quantities of N from the chitin than it did with any other bacteria, whether chitinolytic or not. Moreover, we demonstrated that adding P. pallidum to the hyphosphere with Paenibacillus sp. further increased by at least 65% the gain of N from the chitin by the AM fungus compared to the hyphosphere without protists. We thus directly demonstrated microbial interplay possibly involved in efficient organic N utilization by AM fungal hyphae - and their associated host plants.

SEED TRANSMITTED BACTERIA AND FUNGI DOMINATE JUVENILE PLANT MICROBIOMES

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Plant microbiomes play an important role in agricultural productivity, but there is still much to learn about their provenance, diversity and organization. In order to study the role of vertical transmission in establishing the bacterial and fungal populations of juvenile plants, we used highthroughput sequencing to survey the microbiomes of seeds, spermospheres, rhizospheres, roots and shoots of Arabidopsis thaliana, Brachypodium distachyon, maize, rice, switchgrass, Brachiaria decumbens, wheat, sugarcane, barley, sorghum, tomato, coffee, common bean, cassava, soybean, pea and sunflower. Unsterilized seeds were planted in either sterile sand or farm soil inside hermetically sealed jars and after as much as 60 days of growth, DNA was extracted to allow for amplicon sequence based profiling of the bacterial and fungal populations that developed. Seeds and spermospheres were dominated by Proteobacteria and Ascomycetes, with all containing OTUs belonging to Pantoea, Enterobacter, Pseudomonas, Bacillus and *Fusarium.* All plants grown on sterile sand in sealed jars developed microbiomes dominated by shared Proteobacteria and diverse fungi, with 63 seed transmitted bacterial OTUs present in both sand and soil grown plants. While most mycobiome diversity was observed to come from soil, judging by read abundance, the dominant fungi were also vertically transmitted. A core bacterial microbiome appears to exist, with seed transmitted fungi and bacteria dominating juvenile plant microbial populations. Further study of these seed transmitted microbes will be important to understand their role in plant growth and health, their fate during the plant life cycle and may lead to innovations for agricultural inoculant development.

IN-VITRO SCREENING OF POTENTIALLY SUPPRESSIVE SOIL FROM NATURALLY GROWING BANANA RHIZOSPHERE AGAINST PANAMA WILT DISEASE PATHOGEN

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Fusarium oxysporum f.sp. cubense (Foc) TR1 is one of the dreadful pathogens of bananas that caused the extinction of commercial varieties in the past. In 2020, reports on TR1 isolated from the infected cavendish banana cv. Grand Naine from India raised concern alongside TR4 which forced Colombia to declare a national emergency. Here we explore potentially suppressive soil for sustainable management of Panama wilt. A Total of 41 nos of soil samples from the banana rhizosphere were collected from forests and naturally growing sites and evaluated in-vitro against the pathogen Foc TR1 using solid media and aqueous soil extracts. The pathogen growth suppression recorded in solid media ranged between 0.12-1.48 cm, whereas, in aqueous soil extracts, the measured optical density (OD) laid between 0.074-0.129. Of 41 nos of samples, AUDC (02, 03, 05, 07, 12, 14, 15, 17, 19, and 23) exhibited significant suppression of mycelial growth whereas, AUDC 05 showed the maximum suppression and could be due to the production of natural products by Non-ribosomal peptide synthetases (NRPSs) of bacterial and fungal origin. Besides, samples from natural sites close to urban regions showed minimal suppression, indicating a poor disease suppression microbiome structure, albeit forested. Soil samples that exhibited higher suppression were from the Karbi Anglong and Dima Hasao region of Assam, India, having the highest forest coverage among all the districts. Inundative application of biocontrol could be ineffective in managing the pathogen due to edaphic factors. Therefore, these soil samples may be explored further to help develop effective bioformulations.

TACKLING PLANT STRESS THROUGH METHYLGLYOXAL METABOLIC MACHINERY OF MICROBES

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Root-associated microbes offer immense potential for improving plant growth in an environmentally sustainable manner. However, traits generally studied for assessing growth promoting potential of the microbes are not always or perhaps the only ones, contributing towards plant growth. Thus, it is desirable to inspect more and more genomes in order to identify novel traits and corresponding genes for efficient screening of potential growth-promoting microbes. Towards this, we have analysed the genome sequences of two novel isolates viz. Pseudomonas sp. CK-NBRI-02 (P2) and Bacillus marisflavi CK-NBRI-03 (P3). Our studies not only unravel their growth-promoting potential but also provide a comparative assessment of different plant-beneficial traits, correlating them with differential ability of microbes to alleviate plant stress. Importantly, we found that the microbes also differed in their ability to metabolize methylglyoxal, a ubiquitous molecule which is an important determinant of plant stress. P2 exhibited greater tolerance to MG and therefore, possessed better ability to sustain plant growth under dicarbonyl stress. Whereas under salinity conditions, only P3 exhibited a dose-dependent induction in MG detoxification activity in accordance with concomitant surge in MG levels, contributing to enhanced salt tolerance in plants. Importantly, application of either strain affected MG levels in Arabidopsis and subsequently its detoxification machinery, probably to strengthen plant growth and defence response. Through this study, we propose a critical role of microbial MG detoxification in plant growth promotion under stressful environment and suggest that it serves as a beneficial trait which may be considered while screening microbes for stress mitigation in plants.

A NEWLY ISOLATED PURPUREOCILLIUM SPECIES ENDOPHYTICALLY COLONIZES WHEAT AND PROTECTS IT FROM MAJOR FUNGAL DISEASES

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In nature, soil-borne fungi colonize roots of healthy plants although it's usually unclear whether these associations constitute random encounters or provide actual fitness benefits to the hosts. Fungal pathogens for instance are a serious threat to global food security, sustainable methods are vital to help control these pathogens in the face of a rapidly growing human population. Here, we show how a newly isolated Purpureocillium species *YZ1* endophytically colonizes wheat roots and systematically spreads into shoots without causing any visible disease symptoms. We show that *YZ1* can also grow endophytically in Maize, Millet, and Sorghum and promote their growth in greenhouse conditions. We also show that *YZ1* can significantly promote wheat growth and induce strong disease resistance against Fusarium crown rot caused by *Fusaium graminearum* and Powdery mildew caused *Blumeria graminis* f. sp. Tritici when inoculated in roots.

Together, YZ1 was shown to promote wheat growth and induce strong disease resistance against major wheat diseases. This work provides evidence of how YZ1 can be developed into a novel growth-promoting and biological control agent against Fusarium crown rot. Our work also raises inciting questions on how beneficial root endophytes can systematically spread to the shoots with beneficial outcomes.

BACTERIAL LIPOXYGENASES MAY FACILITATE PLANT-HUMAN AND PLANT-ANIMAL HOST JUMPS

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Lipoxygenases are enzymes that participate in the biosynthesis of oxylipins – oxidized PUFA derivatives. These products perform cell-to-cell signalling functions in multicellular eukaryotes, such as animals, plants, and fungi. Lipoxygenases are also present in bacteria, but the functions of these enzymes remain poorly characterized. Most data is available for *Pseudomonas aeruginosa*, whose lipoxygenase is found to suppress the immune response through host-microbe oxylipin signalling (*Morello et al., 2019, DOI: 10.3389/fmicb.2019.01826*).

In our recently published bioinformatic research, we have found bacterial lipoxygenases to be associated with complex structure formation, pathogenicity and symbiosis (*Kurakin et al., 2020, DOI: 10.1134/S0006297920090059*). Here, I present a follow-up research of the link between lipoxygenases, pathogenicity and symbiosis.

I performed phylogenetic analysis of lipoxygenase sequences belonging to plant symbionts, cross-kingdom (plant/animal) pathogens and animal/human pathogens. I have found that there were at least three independent series of horizontal transfer of lipoxygenase gene, that link plant symbionts, plant/animal pathogens and animal pathogens together, with the good correlation between phylogenetic clustering of lipoxygenases and the ecology of respective bacteria. It means that lipoxygenases are involved in the host-microbe signalling in a wide range of bacteria (such as *Variovorax spp., Pantoea ananas, Burkholderia gladioli, Pluralibacter gergoviae*) in a similar way like in *Pseudomonas aeruginosa*. Many of these bacteria are associated with plants, others are dangerous nosocomial pathogens.

I concluded that lipoxygenases may facilitate cross-kingdom host jumps of bacteria between plants and animals/humans. It could be of concern in relation to phytopathology and emerging bacterial pathogens surveillance.

LIVING MULCH FOSTERS A MORE DIVERSE AND BALANCED BACTERIAL COMMUNITY IN CORN PRODUCTION

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The effect of cover crops on bacterial aspects of soil health has not been extensively examined, especially in the context of year-round "living mulch" cover crop systems. Here, we investigate the effect of a variety of cover crops on the bacterial community of maize fields after four years and compare the differences both among cover crop treatments and relative to a no-cover control. We compared the effect of a living-mulch (LM) perennial white clover (Trifolium pratense L) system, annual cereal rye (Secale cereale L.) (CR), annual crimson clover (Trifolium incarnatum L.) (CC), and no-cover (NC) control sampled three times during the 2018 growing season. 16s rDNA amplicon analysis of the soil bacterial community shows that bacterial community composition in cover crop treatment was significantly different from NC control, and LM, CR accommodate more heterogeneous and even bacterial communities compared to NC control. We also found that the difference in bacterial composition between treatments was driven by soil nitrogen concentration and lime buffer capacity, evidenced by the differential abundance pattern of bacterial taxa responding to these two soil chemical characteristics. Moreover, bacterial diversity also was found to have a significant association with metal ion concentration and nitrogen, and that these associations were both stronger and more numerous later in the season. These results are among the first to look at how a perennial cover crop affects the membership of the bacterial soil community and help advance our understanding of how crops, management, and soil microbiomes interact.

THE EFFECTS OF UREA FERTILIZER ON METHANOGENESIS AND RELATED UPSTREAM METABOLIC PROCESSES IN RICE-ASSOCIATED MICROBIOMES

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Rice cultivation is a major source of anthropogenic methane emissions; approximately 15% of annual methane emissions originate from rice paddies. The addition of nitrogen fertilizer has been shown to affect the rate of methane emissions in many studies, though the results are often conflicting: some studies demonstrate an increase in methane emissions with the addition of fertilizer while others demonstrate a decrease. One possible explanation for these various results is that soils from different geographic locations harbor unique microbial communities, and the addition of nitrogen could affect these communities and functions in a soil-dependent fashion. The unique impacts on different taxa or functions could then subsequently impact the syntrophic relationships with and production of precursor molecules for methanogenesis. To test this, we grew rice in three different soils originating from different rice paddies across northern California, and tested the effect of urea fertilizer on the root associated microbiomes. We identified both shared and soil-specific responses among the fertilizer-affected taxa. We further profiled the shifting abundances of microbial functions in the rhizosphere samples of a single soil through shotgun metagenomics, and found that the high nitrogen treatment samples had a greater abundance of genes associated with methanogenesis pathways, fermentation pathways that produce methanogenic precursor molecules, and phenolic degradation pathways that produce the precursor molecules for the fermentation pathways. These results demonstrate that urea fertilizer increases the abundance of methanogens, and suggests some upstream pathways that could potentially feed into that process.

CAN STEM MICROBIOME DEFINE THE PLANT? TRADITIONAL AND COMMERCIAL TOMATO GENOTYPES EXPOSE THEIR DIFFERENCES

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Plant microbiome is known to have a key role in the behavior of the host plant¹. Alternatively, plant genotype is reported to affect the microbiome composition and structure². Thus, we wanted to understand the relation between the microbiota and the host plant, by studying stem microbiome of six tomato genotypes: four Mediterranean traditional tomato varieties (ADX, TH-30, ISR, MO-10) and two commercial cultivars (Ailsa Craig, Moneymaker). Plant stems were sampled from 4 week old plants grown under controlled conditions. DNA samples were used to perform amplicon sequencing, targeting ITS region for fungi and 16S for bacteria, and posterior bioinformatics analysis was performed in QIIME2 platform and RStudio. Our results strongly suggested that traditional genotypes held a richer microbiome than their cultivated counterparts. Traditional tomato was assigned significantly more taxa, with many unique taxa. In addition, the composition of fungal communities was also more diverse and had a seemingly wider phylogenetic background. These results prove that traditional varieties, which have been subject to lower pressure of manipulation, are not only a richer source of potential beneficial microbiota, but possess bigger diversity than commercial varieties, which could influence the behavior and physiological responses of each genotype.

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THE CONFLICT BETWEEN ANTIBIOTIC PRODUCTION AND RAPID GROWTH IN THE PLANT MICROBIOME

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Microbial communities employ a variety of complex strategies to compete successfully against competitors sharing their niche, with antibiotic production being a common strategy of aggression. Four non-ribosomal peptides/polyketide (NRPs/PKS) antibiotics produced by *subtilis* clade, are produced on the root and determine the results of microbial competition for plant colonization ¹. We revealed that these antibiotics acted synergistically to effectively eliminate phylogenetically distinct competitors. The production of these antibiotics came with a fitness cost manifested in growth inhibition, rendering their synthesis uneconomical when growing in proximity to a phylogenetically close species, carrying resistance against the same antibiotics. To resolve this conflict and ease the fitness cost, NRPs production was only induced by the presence of a peptidoglycan cue from a sensitive competitor, a response mediated by the global regulator of cellular competence, ComA. These results experimentally demonstrate a general ecological concept – closely related communities are favoured during competition, due to compatibility in attack and defence mechanisms ².

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PLANT GROWTH PROMOTING BACTERIA ASSOCIATED WITH ASIAN RICE (ORYZA SATIVA L.) SUBSPECIES INDICA AND JAPONICA HAVE A POSITIVE INFLUENCE ON GROWTH OF ONLY THE JAPONICA SUBSPECIES

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Asian rice (Oryza sativa L.) is one of the most important crops because it is a staple food for almost half of the world's population. O. sativa has two subspecies, JAPONICA and INDICA. Bacteria that are involved in improving plant health, known as plant growth promoting bacteria (PGPB), have beneficial interactions with plants. The main research question of this study is to determine whether both JAPONICA and INDICA will each be positively influenced by same PGPB. To address the question, we initially isolated 140 bacteria from INDICA and JAPONICA root and leaf tissues at their early-stage development (2-week-old plants). The bacterial isolates were first screened for their ability to solubilize phosphate and 25 isolates were positive for this test. We then tested whether these 25 bacteria were able to produce other potentially growthpromoting factors. Five of the most promising bacterial isolates from the INDICA and JAPONICA roots and leaves were chosen for whole genome sequencing. Data showed that these bacteria were members of the genera Paenibacillus, Brevibacillus, Microvirga and Pseudomonas. Among these five bacteria Pseudomonas mosselii, Microvirga sp., Paenibacillus rigui and Paenibacillus graminis, when used to inoculate seeds improved subsequent JAPONICA plants' growth compared to uninoculated controls (P<0.05, T-test). However, these bacteria did not have a similar effect on INDICA growth. This indicates that while bacteria may have several known plant growth promoting functions, their effects on growth parameters can be subspecies dependent and suggest close relationships between plants and their microbial partners.

MAIZE ROOT-ASSOCIATED MICROBES LIKELY UNDER ADAPTIVE SELECTION BY THE HOST TO ENHANCE PHENOTYPIC PERFORMANCE

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Root-colonizing microbes have been shown to promote the growth and development of the host plant. However, it remains largely unknown to what extent the host genome affects root microbial communities. A set of 230 maize (Zea Mays) genotypes drawn from the Buckler-Goodman association panel were grown under standard agronomic practices and nitrogen (N) deficient conditions in a two-year field experiment and the composition of rhizosphere microbial communities was assessed through 16S amplicon sequencing of 3,300 rhizosphere samples. We constructed a functional core microbial community that consists of 150 highly abundant and consistently reproducible microbial groups at the family, genus, and species level. Distinct compositions of microbial communities were observed between genotypes, and between the same genotypes grown under different N treatments. Our analysis suggests that 79 microbial groups (i.e. "rhizobiome traits") are heritable under either or both N treatments and that 34 traits are under adaptive selection. Genome-wide association studies (GWAS) using a genetic marker set of approximately 20 million SNPs identified a set of 467 genetic loci that are strongly associated with the abundance of 115 microbial groups. Integration of RNA sequencing and aerial phenotyping data further revealed that genes near the microbe-associated loci are preferentially expressed in roots, and that the abundance of 62 genome-associated microbes directly correlates with plant performance in the field. A better understanding of these plant gene-microbe interactions may open avenues to sustainably improve crop performance in the agricultural industry.

ARTIFICIAL SOIL SELECTION APPROACH FOR DETERMINING BASELINE COMPOSITION AND FUNCTION OF SOLANUM TUBEROSUM RHIZOSPHERE MICROBIOMES

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Over millions of years terrestrial plants have co-evolved with their root associated (i.e. rhizosphere) microbes to overcome challenges, relying on them for non-innate immunity, stress tolerance, and nutrient acquisition. We found that domestication of potato has influenced the assembly and composition of their associated microbial communities. However, we still lack a framework for predicting the net effect of a given community on its host based on knowledge of its composition. Here we implemented a multi-selection event approach to progressively select and assemble microbial communities with beneficial or detrimental net effects on host growth. Using this multi-selection event approach, we can pick apart the microbial black box in terms of function in tamed and semi-wild potato microbial communities. We found (i) host specific differences in response to differences in microbial beta and alpha diversity and (ii) differences among plant hosts in selecting rhizosphere microbes across time. Our ability to generate consistently beneficial or detrimental microbial communities with varying functional community. Having generated microbial communities with varying functional consequences for host growth, we are now attempting to understand the compositional changes that drove the functional divergence.

DEVELOPMENT OF RNA EDITING SYSTEM BY EXPRESSING HORNWORTS SPECIFIC "DYW-TYPE" PPR PROTEINS

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Pentatricopeptide repeats (PPR) proteins are exclusively act as sequence-specific RNA-binding proteins within mitochondria and chloroplasts in almost all land plants. Genome-wide analysis of the hornworts, Anthoceros agrestis, revealed the PPR proteins in this species contain unique Cterminal DYW-like domains with specific signatures. These domains are the strongest candidates for the U to C RNA editing enzyme, since such domains were not observed in other model plant species having only C to U RNA editing. In present work, we explored the study on three different variants of C-terminal PPR proteins of hornworts, GRP-type, DRH- type and DYWtypes. We have investigated the RNA editing events by cloning the Hornworts PPR genes. An expression system was developed in which the Hornworts specific PPR protein variants were cloned with PPR56 (truncated DYW), Physcomitrella patens (moss) editing factor. The assay system allowed to study RNA editing by hornworts PPR genes with its potential target sequences in bacterial and animal cells. Furthermore, we investigated the expression level of these variants, and our results suggest that the DYW-type domains are comparatively more expressive than the other two variants. Thus, it was concluded that Hornworts PPR proteins containing the Cterminal DYW domains perform similar to that of other DYW domains known for having conserved residue of cytidine deaminase activity, therefore can be enough for efficient C-to-U RNA editing. However, for the "reverse" U-to-C RNA editing, these variants were not sufficient and other unknown RNA editing factors are further need to be investigated.

SEED MICROBIOME OF CEREALS, OILSEEDS, AND LEGUMES

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Microorganisms colonize all plant organs including seeds. Seeds are able to carry and transfer microorganisms from one plant generation to another acting as an initial source of microbial inoculum for plants. These seed-associated microbial communities offer the potential of improving crop production and yield through the protection against abiotic and biotic stresses. Despite their agricultural relevance, seed-borne bacterial and fungal communities as well as factors influencing their assemblage remain largely unknown. In our study we i) characterized the seed-associated bacterial and fungal microbiomes of three important agricultural crops: wheat, canola, and lentil, *ii*) explored genetic and environmental factors influencing the assembly of the microbiota carried by seeds, and *iii*) examined the preservation and transmission of seed microbiomes. Seed samples of different lines harvested from different field locations, years, and generations were subjected to high-throughput amplicon sequencing of the bacterial 16S rRNA and fungal ITS regions. Our results suggest recruitment, transmission, and preservation of seedassociated microbiota are determined mainly by the environment in which the plants are grown and to some extent by the host. In addition, a shared set of microorganisms (i.e., core microbiome) was found when seed microbiomes of different crops, lines, and from different sources were analyzed together. The existence of this core microbiome implies that plants recruit and carry microorganisms that could interact with further generations, affecting their adaptation and establishment in novel environments. Collectively, these findings represent an important step toward the advancement of sustainable breeding and agricultural strategies to utilize microbial communities carried by seeds.

PHYLOGENETIC CONSERVATION OF BIOSYNTHETIC GENE CLUSTERS AMONG PHYTOBIOME

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Specialized metabolites produced by microbes are of immense importance in the field of agriculture, pharmaceuticals, and human health. Importantly, these molecules are ecologically relevant as they mediate interactions between plants and their associated microbes. Microorganisms inhabiting various niches in plants, produce a plethora of secondary metabolites that influence the outcome of plant-microbe and microbe-microbe interactions. Therefore, a predictive understanding of the secondary metabolite biosynthesis potential of the strikingly diverse microbial world within and around the plant host would help elucidate the ecological processes underlying phytobiome-associated processes. Phylogenetic information of microbial traits allows understanding of ecosystem functioning of microbes. Such understanding of biosynthetic gene clusters is currently lacking in phytobiomes. To fill this gap, we studied the phylogenetic distribution of secondary metabolite biosynthesis gene clusters using genomic information from a large collection of both cultivated (isolates) and uncultivated (Metagenome assembled genomes or MAGs) plant-associated microbes. We applied both D-test and consenTRAIT for phylogenetic conservation analysis on both plant-isolates and plant-MAGs to infer the strength and genetic depth of conservation respectively. Our results from both approaches show that biosynthetic gene clusters are highly conserved among plant-associated microbes. Further, consenTRAIT analysis revealed that the mean genetic depth of conservation of these traits are similar to complex microbial traits (e.g., methane oxidation), suggesting the ability to produce secondary metabolites are deeply conserved among plant-microbiome. This study demonstrates that a phylogenetic framework is useful for predicting the potential role of chemical ecology in the functioning of phytobiomes.

RHIZOBIALES COMMENSAL BACTERIA PROMOTE ARABIDOPSIS THALIANA ROOT GROWTH VIA HOST SULFATED PEPTIDE PATHWAY

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Roots grow in soils being surrounded by a variety of microbes, a part of which establishes the root-associated microbial community ("root microbiota"). Root microbiota members referred to as root-associated commensals, are able to manipulate host root development and impact host physiology. We recently reported that the bacterial root commensals that belong to the order Rhizobiales, which also contains symbiotic and pathogenic bacteria, promote primary root growth of Arabidopsis thaliana by facilitating cell division in the root meristematic zone (Garrido-Oter*, Nakano*, Dombrowski* et al., 2018). However, the molecular mechanism underlying this root growth promotion (RGP) activity remained unclear. Here, we conducted a transcriptomic analysis of A. thaliana roots inoculated with root-associated commensal bacteria of Rhizobiales and sister lineages and revealed common and strain/lineage-specific transcriptional response, likely mediated by WRKY and ANAC families of transcription factors. We identified several candidate ANAC family transcription factors that specifically respond to Rhizobiales strains and possibly regulate Rhizobiales RGP. By integrating with developmental and cell biological experiments, we identified a crucial role of host sulfated peptides, whose activity relies on the enzyme TYROSYLPROTEIN SULFOTRANSFERASE (TPST), in Rhizobiales RGP, while none of the known sulfated peptide receptors appeared to be required for this activity. Finally, we show that TPST is needed for RGP exerted by Rhizobiales but not Pseudomonadales isolates, delineating lineage-specific mechanisms to manipulate host root development.

BIOTECHNOLOGICAL POTENTIAL OF BACILLUS VELEZENSIS

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The development of antimicrobial drugs necessary to combat diseases of plants, animals, and humans is one of the promising areas of the pharmaceutical industry and agriculture. Currently, the search and study of new strains of bacteria of the genus *Bacillus*, producing a wide range of biologically active substances and showing antagonistic properties against pathogenic bacteria and fungi, is underway.

Bacillus velezensis is a non-pathogenic species to humans and animals and can enter into mutually beneficial relationships with plants. *B. velezensis* produces enzymes and lipopeptides such as surfactin, iturin, fungicidal antibiotics, and aminoglycosides, which are used in the agro-industrial complex and medicine.

We have determined the antagonistic activity of the *Bacillus velezensis* 13 strain, isolated from the leaf surface of the hawkweed *Hieracium robustum* Fr. s. L., 1848, to a number of strains of gram-positive and gram-negative bacteria and phytopathogenic fungi, the spectrum of enzymatic activity of the strain, and conducted the isolation of the cyclic lipopeptide surfactin preparation. The investigated strain of *B. velezensis* 13 exhibits antagonistic activity against some ascomycetes and gram-positive bacteria, but not against gram-negative ones, which may be due to the specificity of the antibiotics produced. Determining the production of hydrolytic exoenzymes we found out that the *B. velezensis* 13 strain has cellulolytic, proteolytic, amylolytic, β -glucosidase, and lipolytic activities. Our experiments on the detection of compounds of the lipopeptide nature showed that the *B. velezensis* 13 strain is able to synthesize substances that, according to TLC and IR spectroscopy, are representatives of surfactins.

COMPARATIVE GENOMICS REVEALS THE ORGANIC ACID BIOSYNTHESIS METABOLIC PATHWAYS AMONG FIVE LACTIC ACID BACTERIAL SPECIES ISOLATED FROM FERMENTED VEGETABLES

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Lactic acid bacteria (LAB) constitute a widespread bacterial group, inhabiting the niches of fermented vegetables and capable of producing beneficial organic acids. Here, we performed whole-genome sequencing and comparative genomics analysis using several bioinformatics approaches to investigate five LAB species, Lactobacillus plantarum PC1-1, Pediococcus pentosaceus PC2-1(F2), Weissella hellenica PC1A, Lactobacillus buchneri PC-C1, and Enterococcus sp. YC2-6 isolated from fermented vegetables, to enhance our understanding of their different genetic functionalities and organic acid biosynthesis. The results revealed the presence of major carbohydrate-active enzymes, putative operons, and unique mobile genetic elements, which included plasmids, resistance genes, insertion sequences, and putative transposons involved in organic acid biosynthesis, bacteriocin production, and genome plasticity. Besides, their genome arrangements were conserved within gene orders. We also reconstructed their organic acid metabolic pathways, emphasizing the key genes that encode specific enzymes required for organic acid metabolism. In addition, the five genomes were found to contain various regions of secondary metabolites biosynthetic gene clusters, including the T3PKS domain enriched with unique genes encoding a hydroxymethylglutaryl-CoA synthase and a CPR transcription factor with novel binding sites involved in organic acid biosynthesis and capable of exhibiting specific antimicrobial activity, which could enable them to prevail in the fermentation process. Therefore, it is envisaged that the comparative genomic analysis of these LAB species will provide a detailed insight into their potential industrial applications.

THE MOLECULAR BASIS OF IMMUNE-SUPPRESSION BY ROOT COMMENSAL RHODANOBACTER IN ARABIDOPSIS THALIANA AND ITS ROLE IN MICROBIOTA ESTABLISHMENT

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We recently showed that the ratio of immune-suppressive and non-suppressive bacteria in the root microbiota is essential for the balance between plant growth and defense (Ma et al. Nature *Plants* 2021). Immune-suppressive activities were found across all four phyla of the Arabidopsis root microbiota, but were highly conserved within the order Xanthomonadales, which defines one core lineage of the root microbiota. We conducted an in planta forward genetic screen using a transposon insertion library of the Arabidopsis root commensal Rhodanobacter R179, which belongs to the deepest branch of the Xanthomonadales. In comparison to R179 wild-type, R179 defense suppression system (dss) insertion mutants are impaired in their immune-suppressive activity, as seen by reduced root growth upon chronic exposure to immune elicitors and enhanced expression of defense marker genes. Two dss genes characterized in more detail co-occur in most Xanthomonadales genomes, but are mostly absent in the non-Xanthomonadales lineages of the root microbiome. This is consistent with the conservation of immune-suppression in the Xanthomonadales and suggests the existence of other immune-suppression mechanisms in distinct lineages of the microbiota. The activation of plant immunity shapes root microbiota profiles, and we have recently shown, that the composition of exclusively non-suppressive bacterial communities is vulnerable to chronic immune stimulation (Ma et al. Nature Plants 2021). Co-inoculation of wild-type R179, but not the tested dss mutants, with synthetic bacterial communities specifically alters the profile of the non-suppressive community on roots. This supports the immune-suppressive activity of Rhodanobacter R179 dss genes and suggests that these genes stabilize non-suppressive communities in planta.

THE MICROBIOME OF LUPINUS ANGUSTIFOLIUS: A POTENTIAL PROTEIN SOURCE IN EUROPE

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Lupinus angustifolius is a plant that can be used as a protein source for both animals and humans. Lupins are successful protein crops in Australia, yet lupin production in Europe is insufficient to guarantee the stable and sufficient supply required for its use by the food and feed industry. It is essential to describe and understand the functional role of the microbiome associated with a host plant. This information will be useful for agricultural applications.

This work was designed to study the non-culturable microbiome (fungi and bacteria) associated with the rhizosphere and the soil where *L. angustifolius* grows wild. Soil and rhizosphere samples of this legume were collected from two different locations in Spain. In addition, isolation protocols were designed to isolate the culturable bacterial microbiome. The criteria used for the isolation focused on the most abundant taxa detected by metagenomics in a proportion >1%.

The microbiome of *L. angustifolius* varied greatly depending on the physico-chemical properties of the soils sampled. Metagenomic analysis of the soil samples showed that the most dominant bacterial phyla were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Acidobacteria*. In Cabrerizos, the phyla *Ascomycota* and *Mortierellomycota* stood out; while in Salamanca, *Ascomycota*, *Basidiomycota* and *Mucoromycota* were found in a higher proportion. On the other hand, in the rhizosphere samples the bacterial and fungal communities were very similar, confirming that *L. angustifolius* has a very closely associated non-culturable microbiome. The culturable microbiome confirmed that the highest number of strains isolated from both rhizosphere and soil samples matched the most abundant genera in the metagenomic analysis.

DISENTANGLING THE GENETIC BASIS OF RHIZOSPHERE MICROBIOME ASSEMBLY IN TOMATO

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Plant microbiomes play a pivotal role in plant growth and health, but the mechanisms driving microbiome assembly remain largely elusive. Here, taxonomic and genomic features of the rhizosphere microbiome were mapped as quantitative traits of a recombinant inbred line population of modern tomato cultivar (Solanum lycopersicum var. Moneymaker) crossed with its wild relative Solanum pimpinellifolium. QTL analysis revealed significant associations of taxonomic and genomic microbiome features with tomato chromosomal regions, with effect sizes ranging from 3 to 21%. Gene content analysis of prioritized tomato QTLs suggests a genetic basis for enrichment of Streptomyces and Cellvibrio in the tomato rhizosphere, including a 6-Mbp region harboring 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) as well as the terpene synthase alpha-humulene/(-)-(E)-beta-caryophyllene synthase. Furthermore, single nucleotide variation in microbial genes related to metabolism of plant associated polysaccharides, iron, sulfur and vitamins within metagenome-assembled genomes of Streptomyces and Cellvibrio showed strain-specific association with plant QTLs. By integrating 'omics and quantitative genetics, our results pinpoint specific microbial and plant traits involved in tomato rhizosphere microbiome assembly, paving the way for future exploitation of the microbiome as a quantitative trait in plant breeding programs.

STRATEGIC ENGINEERING OF RHIZOSPHERE COMMUNITIES USING A NETWORK-BASED METAGENOMIC FRAMEWORK FOR LINKING MICROBIAL TAXA WITH FUNCTION

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Understanding how specific environmental resources may promote positive plant-microbe associations or suppress negative ones is an important step toward optimizing disease control in the microbiome. Previous work in our lab demonstrated that specific mustard seed meal formulations alter the apple rhizosphere microbiome in a positive manner and effectively suppress replant pathogens. In this study we 1) identified metabolic activities underlying functional differences between "effective" and "diseased" rhizosphere soil systems, 2) linked some of these functions with specific microbial taxa using network-based analyses and 3) tested some of these genomic-based predictions experimentally. We successfully identified a compound which selectively modulated microbial composition in the rhizosphere as predicted. The ability to predict how particular source metabolites may be used to "artificially structure" the indigenous soil microbiome is relevant to managing a host of soil-borne diseases, and goes well beyond the specific model suggested in this study.

METABARCODING-DRIVEN ANALYSIS OF BACTERIAL AND FUNGAL COMMUNITIES OF SUGAR BEET PHYLLOSPHERE

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The plant microbiome harbors a large facet of microorganisms that have diverse traits necessary for existence at challenging habitats such as phyllosphere and are involved in different types of interactions with the plants. Identification of microbial community composition is the initial step in understanding the nature of those interactions and detecting pathogens or potential plant beneficial bacteria.

The main goal of this study was to identify bacterial and fungal communities of the phyllosphere of sugar beet originating from a commercial field in Serbia (Vojvodina).

Amplification and sequencing of the 16S rRNA gene (V3-V4 region) and ITS2 gene region were performed at Novogene (HK) Company. Taxonomic annotation was determined at phylum, class, order, family and genus rank. Alpha diversity analysis of bacterial and fungal communities was evaluated through six indices (Observed-species, Chao1, Shannon, Simpson, ACE and Good-coverage).

Taxonomic annotation discovered 1533 OTUs in bacterial and 216 OTUs in the fungal community of the sugar beet phyllosphere. Relative abundance showed the bacterial community was dominated by Actinobacteria (35%), Proteobacteria (31.3%), Bacteroidetes (7.7%), Firmicutes (6.2%), Cyanobacteria (3.8%), Acidobacteria (3.8%), Thaumarchaeota (3.4%), Chloroflexi (2.8%), Gemmatimonadetes (2.8%) and Verrucomicrobia (1.5%). The fungal community consisted of Ascomycota (53.1%), Mucoromycota (4.6%), Basidiomycota (0.2%) Mortierellomycota (0.2%), unclassified (0.4%) and unidentified taxa (41.5%). The bacterial community of the sugar beet phyllosphere showed higher species richness and diversity than the fungal community.

The obtained results showed that the sugar beet phyllosphere represents a valuable reservoir of microorganisms that could be further examined and exploited to promote plant growth.

PLANT IMMUNE COMPONENTS SHAPING THE PHYLLOSPHERE MICROBIOTA - THE IMPACT OF THE NADPH OXIDASE RBOHD

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The plant microbiota consists of a multitude of microorganisms that affect plant health and fitness. However, it is currently unclear how the plant shapes its leaf microbiota and what role the plant immune system plays in this process. In our study, we tested *Arabidopsis thaliana* mutants with defects in different parts of the immune system for an altered bacterial community assembly using a gnotobiotic system. While higher order mutants in receptors that recognize microbial features and in defense hormone signaling showed significant microbial community alterations, the absence of NADPH oxidase RBOHD caused the most remarkable change in the composition of the microbiota. The *rbohD* knockout resulted in an enrichment of specific bacteria. Among these, we identified *Xanthomonas* strains that act as opportunistic pathogens and colonized wild-type plants asymptomatically but caused disease in *rbohD* plants. Strain dropout experiments revealed that the lack of RBOHD unlocks the pathogenicity of these opportunistic pathogens driving dysbiosis in *rbohD* plants. For full protection, healthy plants require both a functional immune system and a microbial community. Our results show that the importance of the plant immune system in controlling the leaf microbiota.

THE MICROBIOME OF RICE WITHOUT AERENCHYMA

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Rice (Oryza sativa) is a shallow-rooting, semi-aquatic crop responsible for supplying an estimated 20% of daily global dietary energy. Rice is traditionally grown in flooded fields to prevent competition with plants that cannot tolerate flooding. One of the greatest challenges to surviving in the aquatic environment is a lack of oxygen diffusion to root tissues in submerged, low-oxygen sediment. To adapt to these submerged sediments, rice root cortical cells undergo reactive oxygen species dependent programmed cell death under low-oxygen conditions to create the aerenchyma, a cavity-filled cortical tissue that facilitates improved diffusion of oxygen. The enhanced diffusion of oxygen through aerenchyma and, by extension, into the rhizosphere is thought to influence the composition of microbial community members in, on, and surrounding these roots. The identification of an IR64 mutant lacking cortical cells, and thus aerenchyma, provided an opportunity to investigate the influence of aerenchyma on the rice root microbiome. 16S rRNA gene sequencing was performed on the endosphere, rhizoplane, and rhizosphere compartments of field-grown rice. Subsequent analyses revealed a surprising increase in the relative abundance of putative aerobes in the mutant endosphere. Aerobe enrichment may be due to an increased oxygen concentration in roots lacking cortical cells from a concomitant reduction in root oxygen consumption, from obstruction by altered cell-wall composition, or due to altered root exudate quantity and chemistry. Further investigation into the oxygen distribution, morphology, and exudate profile of the aerenchyma-less mutant can establish a reason for the observed differences in microbiome composition and putative functions.

SHARED AND DISTINCT BACTERIAL GENES BENEFITING COLONIZATION OF DISTANTLY RELATED HOST PLANT SPECIES

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In nature, plants recruit a diverse microbial community, the plant microbiome, which is distinct from the surrounding soil community. To understand the forces that shape the plant microbiome we first need to characterize the microbial traits and mechanisms that contribute to plant colonization. Past research on such colonization genes was performed in short-term colonization experiments or in highly synthetic systems. Here, we used barcoded mutant libraries to identify bacterial genes that contribute to the ability of two plant-associated bacteria to colonize monocot and eudicot hosts in a long-term, soil-like system. Among the multiple genes and metabolic modules that we found to contribute to plant colonization, we highlight different efflux systems, iron perception genes, and biotin biosynthesis operons. Importantly, plant colonization was not dominated by a single trait, but rather by dozens of genes and functions contributing to colonization. While many of these colonization of only one host and not the other. Such host-differentiating colonization genes may help to disentangle the observed variation between microbiomes of different plant species and promote the prediction and design of plant species-specific microbiota.

BACKTOROOTS: THE PROTECTIVE ROLE OF THE MICROBIOME OF NATIVE SOILS FROM THE CENTRE OF ORIGIN OF TOMATO

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Plant roots are colonized by diverse communities of microorganisms that can affect plant growth and enhance plant tolerance to abiotic and biotic stresses. If and how plant domestication affected the assembly and functions of the root microbiome remains largely unknown for many plant species. Here, we took a BackToRoots approach for tomato to determine the impact of the microbiome in the native soil habitat of the Andean region in Southern Ecuador, the centre of origin of tomato. Both native and agricultural soils were sampled from this region and used in greenhouse assays to determine the effects of the soil microbiome on leaf tissue damage caused by the endemic sap-sucking insect *Prodiplosis longifila* (Diptera: Cecidomyiidae). The results showed a significantly higher percentage of leaf tissue damage caused by *P. longifila* for wild tomato *S. pimpinellifolium* grown in autoclaved agricultural (16%±3) and autoclaved native Ecuadorian soil (15%±2) than in non-autoclaved agricultural (4%±2) and native (7%±2) soils (ANOVA, p = 7.29e-13). For the domesticated tomato *S. lycopersicum* cv.

Moneymaker, however, no significant differences were found in leaf tissue damage between the autoclaved and non-autoclaved native and agricultural Ecuadorian soils. Collectively, our results suggest that the wild tomato species *S. pimpinellifolium* relies much more than the domesticated *S. lycopersicum* cv. Moneymaker on the soil microbiome for tolerance to the insect *P. longifila*. Currently we are identifying the taxonomic and functional diversity of the native soil microbiome in order to pinpoint microbiome members and underlying mechanisms involved in this protective, aboveground effect on insect herbivory.

THE ROLE OF GENETIC VARIATION IN MAIZE RESPONSE TO BENEFICIAL ENDOPHYTES

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Growth-promoting endophytes offer an exciting future in biofertilizers. There is, however, a lack of research in how the plant host affects an endophyte's ability to promote growth. We set out to quantify how different maize genotypes respond to specific growth-promoting endophytes. We inoculated several genetically diverse maize lines with three different potentially beneficial endophytes: Herbspirillum seropidicae, Burkholderia WP9, and Serendipita bescii. Maize seedlings were grown for 3 weeks under controlled conditions in the greenhouse, where several assessments of growth promotion were measured. We found that several maize lines had significant increases in growth for different phenotypes. We found Herbspirillum seopedicae to increase chlorophyll content, plant height, root length, and root volume significantly in different maize genotypes. Burkholderia WP9 did not significantly promote growth in any lines. Serendipita bescii increased both root and shoot mass significantly for 3 maize genotypes. Realtime qPCR was used to quantify the amount of *Serendipita bescii* in the maize roots, and we found that increased endophyte abundance coincided with increased growth recorded in the greenhouse. Finally, we used linear regression and an ANOVA to parse apart where the variation in phenotype was coming from. Overall, the effect of host genotype on the interaction was low, but several outliers indicate that researchers need to take host genetic variation into account when using growth-promoting endophytes. Detangling these interactions will provide a ripe area for future studies to understand how to best harness beneficial endophytes for agriculture.

INDUCED SYSTEMIC RESISTANCE IMPACTS THE PHYLLOSPHERE MICROBIOME THROUGH PLANT-MICROBE-MICROBE INTERACTIONS

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It is becoming increasingly clear that the plant's microbiome plays an important role in defense against pathogens. This is achieved either by direct microbe-microbe interactions or by microbeplant interactions. One example of the latter is the so-called Plant-Growth-Promoting-Rhizobacteria (PGPR) induced resistance (special forms of it known as Induced Systemic Resistance (ISR)): microbes in the rhizosphere interact with and enhance the plant's resistance against above- and belowground pathogens. We were able to show that beside the model strain Pseudomonas simiae WCS417r (WCS417) the strain Bacillus thuringiensis israelensis (Bti) triggers induced resistance in Arabidopsis thaliana. The resistance induced by both strains relied at least in part on different signaling molecules and pathways. Importantly, both PGPR not only enhanced the immunity of Arabidopsis thaliana by direct microbe-plant interactions, they also induced distinct changes in the leaf microbiome upon elicitation of induced resistance. This microbe-plant-microbe interaction led to selective enrichment of certain bacterial strains in the phyllosphere. Treatment with *Bti* was accompanied by enhanced numbers of *Solimonas terrae* in the Arabidopsis phyllosphere. Also, WCS417 itself proliferated on the leaves of both WCS417and Bti-treated plants, and - in turn - promoted the proliferation of a Flavobacterium sp.. At-LSPHERE Flavobacterium strain Leaf82 displayed 100% sequence identity of its V5-V7 16S rRNA gene region with that of the Flavobacterium., and itself enhanced plant growth and immunity when applied to leaves. Thus, immunity in these forms of PGPR-induced resistance is not only granted by tri-partite microbe-microbe and microbe-plant interactions, but might depend on microbe-plant-microbe interactions.

PLANT-GUIDED DESIGN OF ROOT MICROBIOMES FOR DROUGHT TOLERANCE IN RICE

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Design of microbial communities that elicit specific plant phenotypes is a major challenge that has been advanced in recent years through host-mediated microbiome engineering (HMME). In brief, HMME is a screening method that, other factors held constant, assumes any variation in plant host phenotype is the result of variation in microbiome composition and function. By harvesting only the best-performing microbiomes and using them to inoculate a subsequent cohort of sterile hosts, the community can be iteratively directed towards a phenotypic optimum. Here, we apply a HMME strategy to four different soils (rice field, desert, serpentine, and sterilized calcined clay) to select for communities that confer drought tolerance to rice. We demonstrate that, across multiple selection generations, initially highly diverse, phenotypically suboptimal field soil communities can be both simplified and induced to elicit overall improved growth phenotypes and enhanced performance during drought.

DIFFERENTIAL RESPONSES OF THE RHIZOSPHERIC MICROBIOME STRUCTURE AND SOIL METABOLITES IN TEA (CAMELLIA SINENSIS) UPON APPLICATION OF UREA OR COW MANURE

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The rhizosphere is the narrow zone of soil immediately surrounding the root, and it is a critical hotspot of microbial activity, strongly influencing the physiology and development of plants. By analyzing the composition of the bacterial microbiome and metabolome in the rhizosphere of tea (*Camellia sinensis*) plants, we investigated species composition and its correlation to soil metabolites under three different fertilization treatments (unfertilized, urea, cow manure) during three-time points (spring, early and late summer). Fertilizer or manure application was a major stimulant of the diversity of rhizome-associated bacterial communities, while the sampling time played a more minor yet significant role. The bacterial phyla Proteobacteria, Bacteroidetes, Acidobacteria and Actinobacteria dominated the rhizosphere of tea plants regardless of the sampling time. However, the relative abundance of the enriched bacteria varied under the two different fertilizer/manure regimes. Organic acids and fatty acids were potential regulators mediating the interaction between plants and bacteria in the rhizosphere. Bacteria in the genera Proteiniphilum, Fermentimonas and Pseudomonas in March (spring), Saccharimonadales and Gaiellales in June (early summer), Acidobacteriales and Gaiellales in August (late summer) regulated relative contents of organic and fatty acids. This study documents the profound changes to the rhizosphere microbiome and bacterially derived metabolites under different fertilizer/manure regimes, and provides a conceptual framework towards improved performance of tea plantations.

GRAFTED GRAPEVINE MICROBIOTA ACROSS SPACE, TIME, AND SCION/ROOTSTOCK COMBINATIONS

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Plant microbiomes are not static; rather, they are specialized communities that change dynamically over the life cycle of a plant. A two-stage model has been proposed whereby germination is followed by a period of colonization that establishes a "juvenile microbiome" which is displaced by a more stable community called the "adult microbiome". Perennial species pose challenges to the two-stage model with their extended life cycles; for instance, how do seasonal developmental stages (i.e. fruit set, ripening, and harvest) impact this microbiome, and are patterns consistent across different parts of the plant. Grapevines provide an excellent system to address some of the gaps in our knowledge as they are long lived woody perennials (>20 production years), which are able to be grafted allowing for experiments with multiple rootstock and scion genotypes combinations. This study utilized multiple commercial vineyards across the central valley of California with multiple scion and rootstock genotypes. Our sampling was conducted to cover the 2018 and 2019 growing seasons and designed to collect from multiple compartments from each vine (roots, leaves, and berries) to assess bacterial diversity. We found alpha diversity estimates were dynamic across growing seasons for leaves and berries but stable for roots. Beta diversity analyses showed separation of plant compartments but only the root compartment microbiota were impacted by the collection location. We are currently still working to elucidate the compositional shifts in the microbiota of grapevine compartments across both time and space to better our understanding of the seasonal development of grapevine microbiota.

UNWIRING BENEFICIAL FUNCTIONS AND REGULATORY NETWORKS IN THE PLANT ENDOSPHERE

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Plant endophytes have a largely unexplored functional potential to enhance plant tolerance to (a)biotic stress. Within a collaborative consortium we aim to unravel the beneficial functions of endophytes and the mechanisms involved in the interplay with their host. We aim to:

- 1. Decipher endophytic microbial functions activated upon pathogen infection.
- 2. Disentangle plant regulatory networks activating the endophytic microbiome.
- 3. Unravel the chemistry and physiology of plant endophyte interactions.

To cast light on the interplay between microbial and plant metabolic pathways, we are investigating the pivotal role of plant-derived signals in the control of natural product formation by endophytes.

First results show plant signals-dependent antifungal compounds production in several endophytic bacteria. Knockout mutants are generated to investigate the biosynthetic pathways underlying endophytic protection. In order to link the functional and taxonomic diversity in endophytic communities, we search metagenomes for biosynthetic gene clusters. To include eukaryotic microbes, we developed a classifier that distinguishes eukaryotic from prokaryotic sequences with 99.99% accuracy. Our data will be further described in the poster. Multi-omics integration strategies will be implemented to connect genes and expression patterns

to metabolites playing a crucial role in host-microbe interactions.

Metabolomes from plant roots and endophytes will be investigated to identify compounds associated with the pathogen-activated biosynthetic gene clusters, and a 3D map of metabolites production in time and space will be generated using molecular cartography.

THE DEVELOPMENT OF BACTERIALLY ENHANCED PLANT GROWING MEDIA

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The different components of the root zone (plant, microbiome, and rooting medium) can be engineered to enhance crop production sustainably. The application of plant growth-promoting rhizobacteria (PGPR) can contribute to plant growth enhancement. However, multispecies microbial inocula may be more effective in improving plant performance than single species PGPRs. To this end, in soilless systems, the proper selection of the plant growing medium raw materials plays a crucial role in the effectiveness of bacterial community amendment. Therefore, we investigated the effect of bacterial community inoculation on hydroponic lettuce (Lactuca sativa L.) root-associated bacterial community functioning. The lettuce plants were grown in ten plant growing media with varying raw materials. The results showed that the plant growing medium composition determined plant performance and that successful bacterial amendment was a key driver for improved plant performance. The effectiveness of bacterial amendment depended on the bacterial source, but it also depended on the interaction with the plant growing medium. We found that the raw materials had distinct resident bacterial community structures that were batch-dependent. However, amending a bacterial community to the plant growing media allowed more control over the final root-associated bacterial community structure than a single species PGPR inoculum. Both plant growing medium composition and inoculation affected diversity. These changes in diversity were correlated to plant performance, indicating that a high diversity promoted plant growth. These results support the concept of creating bacterially enhanced plant growing media to maximize root-associated microbiome functioning to secure high plant performance in a hydroponic environment.

MICROBIAL CONTAMINANTS IN CIRCULAR PLANT-DERIVED FOOD AND FEED PRODUCTION SYSTEMS

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The use of chemical pesticides and fertilizers will be reduced in plant production systems worldwide in the coming years. Therefore, agriculture must rely on alternatives to protect plants against biotic and abiotic stresses, to supply nutrients to plants and to sustain soil quality. Manure and other animal-derived products are important alternatives, but there are microbial safety consequences with respect to transmission of human pathogens and antimicrobial resistances to plants. The ecology of these so-called 'microbiome invaders' from animal sources is therefore becoming more important to guarantee safety of the food production chain, to protect human health and to avoid circulation and accumulation of human pathogens and resistant microbes between plant and animal production chains. In the context of modern microbiome research, it is a relevant question how to harness plants from microbial invasions from other ecosystems. Human pathogens, in particular the ones taxonomically related to the class of Enterobacteriaceae such as Salmonella enterica and Escherichia coli, are well adapted to plant environments. From an evolutionary perspective it makes sense that plants act as secondary habitats for these enteric pathogens to persist long enough in or near plants to become ingested by herbivorous grazers. Ecological knowledge about human pathogens in plant production systems must lead to new possibilities for interception in agricultural management strategies to minimize circulation between production systems and to sustain safe and healthy food production.

NON-PATHOGENIC PHYLLOSPHERE-INHABITING ENDOPHYTIC BACTERIA EXHIBIT A STATIONARY PHASE-LIKE EQUILIBRIUM

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Plants are in constant association with a plethora of microbes, the majority of which are commensal and cause no harm to plants. How these non-pathogenic microbes interact with plants is poorly understood as most of current bacterial transcriptomic studies focus on short-term interactions during the transition from artificial media to *in planta* colonization, a situation that may not be frequently encountered in nature. In this study, we embarked upon long-term population and *in planta* transcriptomic studies of commensal endophytic bacteria and compared them to non-pathogenic or effector-triggered-immunity (ETI)-inducing strains of the bacterial pathogen *Pseudomonas syringae*. Our results led to the discovery of multiplication-death equilibrium as a common basis for the shared long-term static population densities of nonpathogenic endophytes, as evidenced by the use of fluorescent division reporters or antibiotics that only targeted dividing bacteria. A comprehensive *in planta* transcriptomic analysis revealed a striking similarity between the transcriptomic features of non-pathogenic *Pseudomonas* syringae in planta to that of bacteria in stationary phase in vitro, a metabolically active physiological state in which the production of adaptive secondary metabolites and stress responses are induced. Interestingly, avirulent P. syringae strains experiencing strong immune responses (i.e., ETI) had an in planta transcriptional response that mimicked that of nonpathogens. We propose that the shared long-term population and transcriptomic features of commensal, non-pathogenic and ETI-eliciting bacteria captured in this study likely reflect the steady physiological state encountered by the bulk of endophytic microbiota —excluding virulent pathogens— in their life-long interactions with plants in nature.

EVALUATION OF AGROBACTERIUM T6SS ANTIBACTERIAL WEAPON IN TUMORIGENESIS AND GALLOBIOME

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Type VI secretion system (T6SS) is deployed by proteobacteria to secrete effector proteins into target cells and engages in pathogenesis or interbacterial competition. Previous studies demonstrated that Agrobacterium tumefacien deploys T6SS to attack closely- and distantlyrelated bacterial species in vitro and in planta. Intriguingly, tumorigenesis was not affected by the loss of T6SS when A. tumefaciens was inoculated on host plants in sterile condition or directly on wounded site. Thus, it remains unknown whether T6SS influences tumorigenesis in natural infection process, or affects the composition of bacterial community inside crown galls. Here, we established a soil inoculation method on wounded tomato seedlings and performed 16S rRNA gene amplicon sequencing to address these questions through the comparison of the A. *tumefaciens* C58 wild-type strain and mutants lacking a functional T6SS (i.e., $\Delta tssL$ and $\Delta tssB$). Based on multiple inoculation trials across seasons, all three strains could induce tumors but the mutants have significantly lower disease incidences. The season of inoculation plays a more important role than T6SS in shaping the gall microbiota (i.e., gallobiome), and the influence of T6SS on the gallobiome composition is evident in summer but not in winter. Several Alphaproteobacterial taxa showed higher relative abundance in gallobiome induced by T6SS mutants, suggesting that A. tumefaciens may exhibit T6SS antagonism to these bacteria. Furthermore, A. tumefaciens wild-type strain showed higher or stable colonization efficiency than T6SS mutants on wounded tomato seedlings or rhizosphere. In conclusion, this study suggested that T6SS of A. tumefaciens may promote tumorigenesis in natural infection process and help A. tumefaciens gain more competitive advantage in plant-associated microbiota.

SYNCOM-SPECIFIC EFFECTS OF NATIVE AND NON-NATIVE COMMENSAL BACTERIA ON THEIR HOST PLANT

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Plant roots accommodate host species-specific bacterial communities. The ecological and molecular mechanisms by which those communities are assembled are still unclear. To study how commensals may have adapted to a plant species, we use synthetic communities (SynComs) of strains from our Lotus japonicus and Arabidopsis thaliana bacterial culture collections to let them compete for colonization of the two hosts in gnotobiotic experiments. We showed that there is host preference of commensal communities, where strains accumulate to higher abundances on their cognate host, and that they have a competitive advantage to invade established communities. To investigate the effect of bacterial adaptation to their host, we analyzed the transcriptional outputs of Lotus or Arabidopsis plants inoculated with their native or non-native bacterial SynComs and observed a SynCom-specific host response. We found clusters of homologous genes in both hosts that were induced upon colonization by their native bacteria, which included known transcriptional regulators of plant immunity. In pathogenicity assays, we found that inoculation with native or non-native strains provides a differential protection against colonization by an opportunistic root pathogen. Imaging studies with fluorescently tagged strains provided insights into colonization patterns. Our findings advance our understanding of microbiota assembly, bacterial host adaptation, and are relevant for the design of successful field inocula.

A FUNCTIONAL ROLE FOR HOLOBIONT THEORY IN AGRICULTURE

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Large-scale agricultural practices are major contributors to climate change and biodiversity loss. Modern technologies and practices, such as GM crops and precision agriculture, are helping decrease the amount of pesticides and fertilizers applied to fields, thereby reducing their negative environmental impacts. Other practices, such as regenerative agriculture, aim to build soil health by employing a diverse crop rotation, the use of cover crops, and conservation tillage, which also reduces the need for pesticides and fertilizers. An integration of all available technologies and practices is needed to improve agricultural sustainability while continuing to produce enough food for a growing population. However, a novel paradigm is needed to continue optimizing crop yields while maintaining soil health. We propose that the concept of the holobiont, while theoretically useful for understanding a host and its associated microbiome as a unit, can have a functional purpose in agriculture by monitoring and helping to understand plant and soil health. The holobiont can serve as a unifying theme when integrating available technologies and practices. Considering crops as holobionts provides a framework to incorporate systems thinking across multiple scales, from host-microbiome interactions to agro-ecosystems. Holobiont theory should be central in management practices to optimize nutrient cycling, pest and disease management, and ultimately soil health. This concept can also guide future breeding programs as well as microbial inoculant formulations. A holistic view has the potential for making agriculture more sustainable and resilient in the face of a changing climate.

SPEAKER ABSTRACTS

Abstracts appear in this book in presentation order. Below is an alphabetical list of speakers and the corresponding page for their printed abstracts. The scientific organizers have selected the noted Short Talk (ST) presenters, who were chosen from our abstract submissions.

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