

Book of Abstracts – Oral presentations

Session 1: Plant-microbiome interactions for sustainable agriculture

Microbiome Engineering for a Sustainable Agriculture

Marcel G.A. van der Heijden^{1,2} (*Invited speaker*)

Microbes play a key role in ecosystems. Many microbes are beneficial to plants and ecosystems. It is, thus, of utmost interest to understand whether it is possible to engineer the microbiome in agricultural systems and specifically promote beneficial microbes. In order to test this, we performed large scale field inoculations with arbuscular mycorrhizal fungi (AMF), a group of beneficial soil fungi that can improve plant nutrient uptake and associate with the majority of land plants, including most agricultural crops. We observed that field inoculation with AMF can promote plant yield (significant maize yield increases of up to +40% were detected). AMF inoculation was especially successful in fields where pathogenic fungi were present indicating that AMF reduced disease or enhanced plant fitness. We further observed that the beneficial impact of AMF on plants is linked to hyphal associated bacteria and a range of observations indicate that in fact a tripartite mutualistic association between plants, fungi and bacteria drives the success of the mycorrhizal symbiosis. Microbiome engineering is also possible by altering agricultural management including crop rotation, tillage and land use and we provide a number of examples demonstrating how this can be achieved. Finally, by investigating over 250 sites across 27 countries in Europe we observed a positive link between soil health and plant productivity, indicating that it is beneficial for farmers and land managers to invest in soil and microbiome management.

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Natural and Agricultural Laboratories: Microbiome Applications and Fundamental Knowledge

Antonio León Reyes (*Invited speaker*)

Towards defining the recruitment and ecological function of *Bacteroidota* in the plant microbiome

Ian Lidbury

The phylum Bacteroidota is an emergent contributor towards plant and soil health. Members of this phylum are highly enriched in the plant microbiome; however, they appear to be disappearing from modern day crop varieties. Importantly, the abundance of Bacteroidota, either pre- or post-exposure to various pathogens correlates with the degree of disease incidence. Our lab has also revealed Bacteroidota are key players in transforming organic phosphorus into plant available phosphate. Their enrichment in plant microbiomes strongly suggests Bacteroidota are competitive for nutrients associated with this niche. However, the mechanisms enabling Bacteroidota to competitively acquire nutrients, such as organic

phosphorus and carbon, are unknown. Hence, the plant-microbe or microbe-microbe interactions underpinning their success and functional role are also largely unknown.

Our lab is focused on investigating the ecological function of plant Bacteroidota and the underlying molecular mechanisms driving important plant-microbe-microbe interactions. Using *Flavobacterium* and *Chitinophaga* spp. as the models, we combine comparative omics approaches with bacterial genetics, protein biochemistry, biophysical approaches, light microscopy, and structural biology to uncover the role of Bacteroidota in phospholipid and polysaccharide utilisation. We are focusing on the utilisation of soluble plant hemicelluloses, such as xyloglucan, heteromannans, and heteroxylans. Hemicelluloses are major components of the cell wall, and more recently, high molecular weight root exudates. In addition, our work has revealed plant Bacteroidota possess unique gene clusters to capture and hydrolyse plant phosphatidylinositols, which are important cell signalling molecules regulating immunity, biotic interactions, homeostasis, and development. Interestingly, we observe parallels in how Bacteroidota capture and import polysaccharides and phospholipids. As well as delving into these exciting mechanisms and their contribution towards rhizosphere competence, the link between these metabolisms and plant health will also be discussed.

polysaccharides; phospholipids; Bacteroidota; bacterial genetics; TonB-dependent transport; *Flavobacterium*

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Plant responsiveness to Microbiota feedback

Klaus Schläppi (Invited speaker)

Plant microbiomes, analogous to the gut microbiomes of humans, support their host with vital services such as promotion of growth, nutrition and health. Plants secrete a wide range of exudate compounds from their roots to the rhizosphere, thereby not only feeding the microbes but also shaping their microbiomes. Benzoxazinoids (BXs) are a group of bioactive secondary metabolites that are abundant in root exudates of cereals, including crops like maize and wheat. Exudation of BXs selectively structures the maize rhizosphere microbiota, which then feeds back on a next generation of plants affecting their growth and health. Little is known whether such microbiome feedbacks operate in the field, i.e. impacting agricultural productivity and food quality in the context of crop rotations. In field work with wild-type and benzoxazinoid-deficient maize mutant plants, we assessed performance, yield and food quality of winter wheat in the subsequent crop generation. This work provided evidence that BX-feedbacks can increase yield under agronomically realistic conditions. However, it emerged that not all plant genotypes were able to respond the same way, what pointed to genetic variation in plant responsiveness to microbiota feedbacks. To date, how plants perceive soil microbiomes and mediate their feedbacks remains a black box. In this presentation, I will provide an update on our efforts to uncover underlying mechanisms of plant responsiveness to microbiome feedbacks

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Synthetic microbial communities for disease suppression in hydroponic systems

Samuel Wilkinson, Harry Wright, Tony Ryan, Jurriaan Ton, Stephen Rolfe (*Invited speaker*)

In soils, the complex rhizobiome community interacts with plants in a mutually beneficial manner. Community members can stimulate plant growth and provide protection against disease through direct mechanisms such as antibiosis or competition, as well as indirect mechanisms such as Induced Systemic Resistance (ISR). However, such advantageous interactions are lost in hydroponic systems. In the H3 (Healthy Soils – Healthy Food – Healthy People) project we aim to bridge this gap by developing synthetic microbial communities (SynComs) that can be deployed in hydroponic systems to provide durable and sustainable health benefits to plants. We employed a high-throughput, laboratory-scale system using tomato grown hydroponically in rockwool, to screen a panel of bacteria and fungi, alone and in combinations of up to 3 members, for growth promotion, and protection via ISR against a foliar pathogen (bacterial speck – *Pseudomonas syringae*). Two different SynComs were identified that reproducibly generated ISR in these systems. Subsequently, a commercial-scale glasshouse trial was then used to evaluate the performance of these SynComs in tomato production. We assessed plant growth, impacts on pollinators, fruit yield and quality, as well as post-harvest disease resistance (grey mould – *Botrytis cinerea*). Samples were collected from across the greenhouse at the end of the 6 month trial and used for high-throughput DNA sequencing to determine how SynCom membership changed, spatially and temporally. We are also exploring how rockwool can be replaced with novel growth substrates tailored to enhance beneficial plant-microbe interactions in hydroponic systems and which can be re-used sustainably or recycled at the end of the production runs.

Synthetic communities, SynComs, plant disease, hydroponics, rhizobiome

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A walk on the wild side: exploring the functional potential of Andean soil microbiomes to enhance tolerance of potato to late blight disease

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Plant domestication is a complex process affecting plant genetics, plant phenotype and habitat relocation, collectively referred to as the domestication syndrome. How these changes have impacted the plant microbiome and its functions is largely unknown for most species. In this study, we investigated the effect of domestication on the potato microbiome and its tolerance to late blight disease caused by *Phytophthora infestans*. For the experimental work, we collected native and agricultural soils from 14 sites along the Ecuadorian Andes and grew potato plants (*Solanum tuberosum* group *Andigena* var. *Superchola*) in these soils. After challenging the plants with *P. infestans*, we found that those grown in various native soils exhibited enhanced tolerance to late blight compared to those grown in agricultural soils. Using heat-treated soils and soil microbiome transplantation, we differentiated the contribution of the microbiome from the edaphic factors. We found that only the plants grown in treatments supplemented with 10% live native soil exhibited the disease-suppressive phenotype. Using 16S and ITS amplicon sequencing from bulk soil, rhizosphere and roots, we identified the depletion and enrichment of specific microbes in the agricultural soils across all 14 sites. Further, we were able to pinpoint specific bacterial and fungal taxa associated with late blight tolerance of potato grown in the native soils, referred to as ‘the missing plant microbes’. Our

results demonstrate the potential of the native soil microbiome in enhancing crop resilience to a specific disease.

Domestication, native soil microbiome, rhizosphere, potato, late blight, Ecuadorian Andes

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Session 2: Plant-microbiome communication and assembly

Roles of dispersal and intra-specific competition in the phytobiome

Joy Bergelson (*Invited speaker*) [Cancelled]

I will describe two projects that address understudied topics important to the phytobiome. The first is a study by my former graduate student, Caroline Oldstone-Jackson, demonstrating tremendous variation in the rates at which bacteria move through the soil. The need to travel to a host plant impacts the resultant community structure of microbes associated with *A. thaliana*, when compared to communities that establish without the need to travel. Interestingly, it appears that microbial communities which develop along the path to the host plant create biological barriers, preventing some species from reaching the host. This demonstration of priority effects shaping community structure suggests a strong contribution of species interactions. The importance of within, rather than between-species, competition is explored in the second study, performed by a postdoctoral associate in my laboratory, Andy Gloss. In this study, a competitive hierarchy among strains of *Pseudomonas viridiflava* is revealed. The ability to predict growth of strains is vastly improved by considering the identify of a co-infecting strain, suggesting that these interactions are strong enough to impose selection. GWAS mapping of the impact of 230 *P. viridiflava* strains on one focal *P. viridiflava* strain identifies about 12 candidate genes involved in strain level interactions. One will be discussed as an example. Given that *P. viridiflava* is a very common member of the *A. thaliana* associated microbiome, it appears that strain level competition is a strong selective force in this system.

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Rhizosphere immunity and its precision enhancement

Zhong Wei (*Invited speaker*)

Seed aging affects seedling development and the microbiome of *Brassica napus*

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Seed quality and vigor are highly affected by seed production, storage conditions, and seed aging. The effect of seed aging on seedling development is well known, however, the impact on the seedling microbiome is still poorly understood. As the seed microbiome is the primary microbial inoculum for the plant microbiome, seed microbes are key components for the assembly of the plant microbiota, as well as plant growth and health. Here we investigated the impact of seed aging on the seedling microbiome of four different *Brassica napus* genotypes

originating from two different field sites. Moreover, we explored different seedling phenotypes according to their germination behavior. Seeds received an accelerated aging stress, with higher temperature and humidity for 24 hours. Oilseed rape seedlings were observed to germinate abnormally due to the accelerated aging stress, compared to the control seedlings. Amplicon sequencing of 16S rRNA genes revealed a strong impact of the aging treatment on the seedling microbiome, irrespective of the genotype, location or seedling phenotype. Further, for one of the locations, a significantly reduced bacterial diversity was observed for aged seedlings. Aged seedlings showed a higher relative abundance of Firmicutes (Bacillus, Paenibacillus), whereas Proteobacteria (Pseudomonas, Pantoea) were more abundant in the control treatment. A network analysis revealed stronger correlations for the control treatment than for the aged seedlings, and showed further Bacillus as important node in the bacterial network of aged seedlings. Following these results, bacteria were isolated from germinated seedlings. Isolates of aged seedlings belonged mainly to Bacillus species, and they revealed a stronger siderophore and phosphate solubility performance in vitro compared to isolates of the control treatment. The results support the important role of the seed microbiome for enhanced seed vigor and plant health, and may support strategies for improved abiotic stress management in crops.

seed microbiome, oilseed rape, abiotic stress

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Bacterial gene clusters for the catabolism of plant specialized metabolites are crucial for plant-microbe interactions

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Plant-specialized metabolites (PSMs) secreted into the rhizosphere play crucial roles in modulating rhizosphere microbiota. We previously reported daidzein, a major isoflavone secreted from soybean root, shapes the soybean rhizosphere bacterial community by enriching Comamonadaceae. We also found that α -tomatine, a steroidal glycoalkaloid secreted from tomato roots, and nicotine secreted from tobacco roots enrich Sphingobium and Arthrobacter, which are abundant in the rhizosphere of tomato and tobacco, respectively. We isolated bacteria belonging to these taxa from PSM-treated soil or rhizosphere and found they could degrade these PSMs. We aimed to identify the gene cluster involved in isoflavone catabolism in Comamonadaceae and clarify how bacterial metabolic capacity is involved in the interaction between plants and rhizosphere bacteria. *Variovorax* spp. belonging to Comamonadaceae isolated from soybean root degraded isoflavones. We analyzed their whole genome and transcriptome following daidzein treatment and selected candidate genes involved in the catabolism of daidzein. By the analysis of these candidate genes by generating the disruption mutants and heterologous expression in *E. coli*, we identified an isoflavone catabolism gene cluster, named ifc. Pan-genome analysis revealed that ifc genes are frequently found in bacterial strains isolated from legume plants, and contribute to the detoxification and/or utilization of isoflavones. Gene clusters for the catabolism of PSM were also recently found in bacteria isolated from tobacco, tomato, and maize rhizospheres, suggesting that these gene clusters are widely involved in plant-microbe interactions in the rhizosphere.

Catabolism, Gene cluster, Isoflavone, Rhizosphere, Soybean

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Towards an understanding of root microbiome assembly in model plants and crops: findings from studies using complex bacterial communities

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*These authors contributed equally to the presented work

The complex microbial communities that associate with healthy plants (microbiota) constitute a tremendous, untapped resource for sustainable agriculture. Members of these communities can improve host fitness by increasing nutrient availability, conferring pathogen resistance, and providing resilience to abiotic stresses. Numerous advances in recent years have deepened our knowledge of the composition of host-associated microbial communities but the role of the plant host in structuring microbiota is poorly understood. We have established large culture collections of bacteria associated with different plant hosts and performed reconstitution studies to disentangle the effect of the host on microbiota assembly. In this presentation, I will provide an update on our results from these large studies and provide an outlook into possibilities emerging from our findings.

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Identification of specialized root exudates associated with microbiome assembly of wild and domesticated tomato

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Domestication of plant species has significantly impacted on the rhizosphere microbiome composition, but the underlying chemistry of microbiome assembly in wild and domesticated plant species remains largely elusive. Here, we collected and identified water-soluble root exudates and root volatile compounds (VOCs) from 4 wild and 4 domesticated tomato species and analyzed their chemical composition via untargeted LC-MS/MS and GC-MS analyses. Secondly, we repeatedly inoculated the extracted root exudates into soils from the center of origin and from the center of tomato production to determine specific shifts in the soil microbiome. Thirdly, we deployed a transparent soil system to investigate root colonization and root metabolite degradation of three bacterial species in wild and modern tomato rhizosphere. *Streptomyces arenae* was found to be more associated with modern tomato rhizosphere, while *Cellvibrio* and *Sphingobium* spp. prefer colonizing the wild tomato rhizosphere. First, we showed that the different tomato genotypes have distinct rhizosphere microbiome composition when grown in greenhouse production soil and native soil. Moreover, root exudate composition was distinctly different between wild and modern tomato genotypes, with specific mass features significantly more abundant in the root exudates of the wild tomato species. Preliminary results showed that *Streptomyces* colonized better the rhizosphere of domesticated tomato, while *Cellvibrio* was more abundant in the wild tomato rhizosphere. LCMS/MS analysis further revealed that *Cellvibrio* can better cope with specific root exudate metabolites produced by wild tomato species. Collectively these results suggest that tomato

plant specialized metabolites constitute an important driver for recruiting specific microbial taxa. By integrating ‘metabolomics’ and ‘microbiomics’, we are now investigating and validating which specialized metabolites are key compounds in tomato domestication and the concomitant change in microbiome assembly.

Specialized root exudates, Microbiome assembly, Plant domestication

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Session 3: Technological advances and translation

Deciphering the chemical language of plant-microbiome interactions

Marnix Medema (*Invited speaker*)

Microbial specialized metabolites are important mediators of molecular interactions between microbes as well as with their plant, animal, or human host. In a way, they constitute the ‘chemical language’ of the microbiome. Interpreting this language is key to understanding the mechanistic basis for many microbiome-associated phenotypes, such as disease suppression or growth promotion.

Genome sequence data has revealed that only a tiny fraction of the chemical diversity of these natural products has been unearthed. In recent years, a range of computational methods have been developed to identify these molecules and the metabolic gene clusters that encode their production, and to assess their biological activities. Here, I will highlight recent work performed in my research group on developing and applying these approaches to accelerate natural product discovery, as well as to study the roles of these pathways in microbe-microbe and host-microbe interactions in microbiomes. Specifically, I will highlight new technologies to predict functions of unknown biosynthetic gene clusters, as well as new tools to predict root exudate catabolic pathways and their roles in root colonization by the microbiome.

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Intra-bacteria competition determines plant-beneficial potential of SynComs

Kathrin Wippel (*Invited speaker*)

Plant roots accommodate host species-specific bacterial communities. Moreover, commensal bacteria display host preference when in competition for colonization of their host roots of origin. Identifying the factors controlling this preferential colonization and elucidating the benefits for the host remains an active field of research. We have shown previously that *Arabidopsis thaliana* (At) distinguishes between native At- and non-native *Lotus japonicus* (Lj)-derived bacterial synthetic communities (SynComs) on a transcriptional level, which includes immune-related responses. To explore potential benefits of this specific recognition, we tested if priming with different SynComs results in differential resistance of the host against detrimental microbes. I will show how we used in vitro and in planta assays and community profiling to explore which bacteria play a significant role in host protection against an opportunistic *Pseudomonas* strain. In brief, we found that, among *Pseudomonas* species, direct antagonism and competition affect community structure and can be determinants of

successful pathogen inhibition. The processes are depending on the host plant, which has important implications for beneficial SynCom design. Furthermore, I will discuss in general the relevance of using tailored microbial communities to investigate plant-microbe and microbe-microbe dynamics, argue for the importance of integrating the findings with natural community data, metabolomics, and genomics, and point out important considerations for experimental design.

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baCLIFE: a user-friendly computational workflow for genome analysis and prediction of lifestyle-associated genes in bacteria

Guillermo Guerrero-Egido, Adrian Pintado, Kevin M. Bretscher, Luisa-Maria Arias-Giraldo, Joseph N. Paulson, Herman P. Spaink, Dennis Claessen, Cayo Ramos, Francisco M. Cazorla, Marnix H. Medema, Jos M. Raaijmakers & Víctor J. Carrión

Bacteria have an extensive adaptive ability to live in close association with eukaryotic hosts, exhibiting detrimental, neutral or beneficial effects on host growth and health. However, the genes involved in niche adaptation are mostly unknown and their functions poorly characterized. Here, we present baCLIFE (<https://github.com/Carrion-lab/baCLIFE>) a streamlined computational workflow for genome annotation, large-scale comparative genomics, and prediction of lifestyle-associated genes (LAGs). As a proof of concept, we analyzed 16,846 genomes from the Burkholderia/Paraburkholderia and Pseudomonas genera, which led to the identification of hundreds of genes potentially associated with a plant pathogenic lifestyle. Site-directed mutagenesis of 14 of these predicted LAGs of unknown function, followed by plant bioassays, showed that 6 predicted LAGs are indeed involved in the phytopathogenic lifestyle of Burkholderia plantarii and Pseudomonas syringae pv. phaseolicola. These 6 LAGs encompassed a glycosyltransferase, extracellular binding proteins, homoserine dehydrogenases and hypothetical proteins. Collectively, our results highlight baCLIFE as an effective computational tool for prediction of LAGs and the generation of hypotheses for a better understanding of bacteria-host interactions.

Bioinformatics, Genome analysis, Bacterial Lifestyle

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Gamification of microbiome research dissemination: a video game and the “Microbiome & Health” MOOC

Matthias Schweitzer, Maximilian Wlasak, Florian Marcher, Christian Poglitsch, Tomislav Cernava, Birgit Wassermann, Johanna Pirker, Gabriele Berg

Microbiome research is vital for public health with its potential to provide holistic and sustainable solutions for improving health of humans, animals, plants and ecosystems in alignment with the One Health approach. The composition of the human microbiome was found to be associated with health and disease, and is largely shaped by daily lifestyle choices such as diet and exercise. To raise awareness, we must communicate microbiome research findings to society. We decided to develop a massive open online course (MOOC) and a video game to innovatively teach about microbiomes. We conducted an extensive literature research

and translated all the information gathered in a way that both the public and the academic community can benefit from. The “Microbiome & Health” MOOC (<https://imoox.at/course/microbiome>) offers: i) a definition of the microbiome, ii) an insight into techniques and methods, iii) latest findings in plant microbiome research, iv) facts about the human microbiome, v) knowledge about the resistome and the exposome, and vi) the potential of microbiome research for planetary health and the sustainable development goals. The video game allows learning about the development of the human microbiome, from before birth to death. In scenarios, the player will make lifestyle choices that were shown to affect the human microbiome and thus learn about potential drivers. The quality of this educational game was evaluated using a knowledge questionnaire and feedback surveys. Participants who played the game got on average 62.6% of knowledge questions right, significantly more than participants who didn't play the game (45.5%). Gamification is a growing trend in education and could allow us to reach a different type of audience, and together with the “Microbiome & Health” MOOC, we hopefully can reach a broad audience in both casual and educational settings.

Gamification, Microbiome research dissemination, MOOC, Educational game

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Microdialysis as a tool to study plant microbial interactions

Sandra Jämtgård^{1,2} (*Invited speaker*)

The rhizosphere is a critical microenvironment that plays key roles in plant nutrient availability, largely due to root interactions with rhizospheric microbes. Suitable methods are lacking that elucidate mechanisms determining rhizospheric community structure and function within the context of a dynamic, undisturbed soil. I will demonstrate the potential of a novel experimental system using microdialysis to examine how root exudates affect rhizosphere fungi and bacteria. Microdialysis has been used for low intrusive soil nutrient sampling at the scale of a fine root, with small probes that also enable release of defined compounds. My research group has evaluated whether microdialysis could simulate exudation, by the release of sucrose, and stimulate changes in a soil microbial community, allowing determination of microbes that responded to exudation. Microdialysis successfully stimulated growth on probe surfaces of fungi and bacteria, which were extracted and sequenced for identification. I will present the uniqueness of using microdialysis to study microbial interactions and the possibility to not only release a compound of interest, but to also follow the resulting effect on microbial community composition and activity. In practise, this means that simultaneous release and sampling of chemical responses is possible, with microbial exudates and nutrient availability analysable.

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Seed tuber microbiome is a predictor of next-season potato vigor

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Potato vigor, an important agronomic trait, is heavily influenced by the field of seed tuber production. Soil microbiota vary significantly between fields, impacting plant health and crop yield. Our study demonstrates that seed potato vigor can be predicted based on microbiota associated with seed tuber eyes, the dormant buds that grow out in the next season. By combining time-resolved drone-imaging of potato crop development with microbiome sequencing of seed tuber eyes from 6 varieties produced in 240 fields, we established correlations between microbiome fingerprints and potato vigor parameters. Employing Random Forest algorithms, we developed a predictive “Potato-Microbiome Informed” model, revealing variety-specific relationships between seed tuber microbiome composition and next season’s potato vigor in trial fields. The model accurately predicted vigor of seed tubers to which the model was naïve and pinpointed key microbial indicators of potato vigor. By connecting variety-specific microbiome fingerprints to crop performance in the field, we pave the way for microbiome-informed breeding strategies.

Seed tuber, microbiome, potato vigor, prediction, machine learning

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Session 4: Transfer to and importance of the plant microbiome for other organisms

The edible microbiome: tracking the food-gut axis

Gabriele Berg^{*1,2,3} (*Invited speaker*), Wisnu Wicaksono¹, Tomislav Cernava¹, Birgit Wassermann¹, Ahmed Abdelfattah²

Plant microbiomes are key components for ecosystem health in terrestrial ecosystems. The plant microbiota is vertically transmitted by seeds and replenished horizontally from soil. All plants are holobionts and form a functional unit with its microbiome. Plant diversification and co-evolution shaped the plant microbiome and designed their specific composition and functional interplay including natural biocontrol of pathogens. Human activities in the Anthropocene are linked to a significant shift of diversity and evenness of the plant microbiota, which is also characterized by a decrease of host specificity and symbionts, and an increase of r-strategic microbes, pathogens, and hypermutators. This requires targeted microbiome management for plant and planetary health.

Recently, we have proven the “the edible plant microbiome” by showing the occurrence of fruit and vegetable bacteria in the human gut (Wicaksono et al. Gut Microbe 2023). The microbiomes of fresh fruits and vegetables and the human gut are represented by members in common such as Enterobacterales, Burkholderiales, and Lactobacillales. Plant based diet via fruit and vegetable consumption potentially has a beneficial impact on the functional diversity of gut microbiota particularly due to the presence of putative health-promoting genes for the production of vitamin and short-chain fatty acids. In the human gut, they were consistently present, although at a low abundance, approx. 2.2%. Host age, vegetable consumption frequency, and the diversity of plants consumed were drivers favoring a higher proportion. Overall, these results provide first links between the human microbiome and the environmental microbiome.

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Plant to gut microbiome crosstalks: implications for human health

Hilde Herrema

Composition and function of the human gut microbiome, and thereby human health, is heavily determined by environmental factors. It is for example constantly exposed to ingested microbes. Food-derived microbes such as lactic acid bacteria can even become residents of the human gut. Since the soil and crop microbiome will at least partially be transferred to the gut; these might have an impact on human health. This has hardly been investigated. Herrema will elaborate on microbiome interventions in humans and how the soil to gut transfer might impact human health.

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Plant peptides as new eco-chemicals: an innovative approach to boost a healthy microbiome

Valeria Castaldi, Martina Chiara Criscuolo, Wisnu Adi Wicaksono, Gabriele Berg, Rosa Rao

Keeping up with new, sustainable approaches that might ensure the health of crop plants becomes challenging in the current context of intense environmental changes. Therefore, it is intriguing to investigate the natural stress response mechanisms found in plants while drawing inspiration to improve them. For instance, since the employment of plant-derived peptides as plant resistance inducers is becoming an established reality, it is necessary to look at their impact at multiple levels, microbiome included. In this study, a promising short peptide derived from tomato Prosystemin defense protein was further examined for its impact on tomato phyllosphere microbiome, aiming to determine whether the peptide effect on host defences involves also a shift in the microbial community. The trial considered common agricultural treatment practices, in which resistance inducer molecules are applied to plants through spray delivery every couple of weeks from the submergence of the plantlets until two months of growth. Through a shotgun metagenomics approach, here we show that the peptide treatment cause a shift in the bacterial community, reflected by a decreasing bacterial diversity as well as a specific selection of taxa. Indeed, the performed differential abundance analysis on peptide-treated plants revealed an increase in microbial genera such as Acinetobacter, Sphingobium, Sphingomonas, Brevundimonas, Massilia. These genera are known to help them in terms of stress responses and/or growth performance. Interestingly, such shift is further supported by bacterial networks visualization, where the control is characterized by a dense single network and the peptide has instead two ordered networks, one of which is mainly characterized by above mentioned peptide-enriched taxa. Current functional profiling focusing on nutrient uptake and microbial metabolism may provide additional insights. Overall, beyond the indirect activity against tomato insects and necrotrophic fungi, Prosystemin peptide shows additional positive properties also on a microbiome scale.

Phyllosphere microbiome, small peptides, plant defense, shotgun sequencing, crop protection

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Designing efficient synthetic microbial communities for enhanced survival and efficacy in mitigating biotic stress in agriculture

Rashi Tyagi, Shilpi Sharma

Biotic stress caused by pests and pathogens leads to a massive decline in crop productivity. Further, the alteration in plant physiology associated with climate change has severely affected plant resistance leading to the advent of more virulent strains of phytopathogens. The indispensable role of microorganisms in stress mitigation and augmentation of crop productivity has been known for decades. However, the conventional bioinoculants have not been fruitful in the long run due to reduced persistence under natural conditions due to competition from resident flora. These limitations spawned rhizospheric engineering, which restructures the microbiome to benefit plants by stress mitigation and growth promotion, whilst maintaining the interactions between microbial members. The current study utilizes the bottom-up approach of rhizospheric engineering by generating robust synthetic microbial community/ies (SMC/SynCom) to mitigate biotic stress (*Fusarium udum*) in *Cajanus cajan*. First, a comparative microbiome analysis was done between *Fusarium*-infested and pathogen-free soil using amplicon sequencing to characterise the pathobiome associated with *Fusarium* wilt. Next, a culture bank of bacterial strains isolated from the rhizospheric soil of host plant, antagonistic to *Fusarium*, whilst exhibiting various plant growth-promoting traits was generated. A novel strategy was adopted to check enhanced biocontrol properties exhibited by bacterial strains when present with other resident rhizospheric microbial communities, and the best-performing strains were selected for the constitution of SMCs. The efficacy of these generated SMCs was assessed in vitro through leaf disc and seed germination assays followed by their effect on plant growth attributes and stress markers in planta. The strategic, successful utilization of biocontrol potential of indigenous microbes already adapted to the host environment in eradication of *Fusarium* wilt in pigeonpea would pave the way for utilizing plant-stress-specific microbiomes to counteract other stress conditions in a variety of crops leading to a rise in agricultural productivity.

Rhizosphere engineering, *Fusarium* wilt, *Cajanus cajan*, Microbiome, Sustainable agriculture

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Expansion of herbivory, constriction to a host plant: a symbiont's work

Ana Simao Pinto de Carvalho (*Invited speaker*)

Insects exploit a great variety of often very specific diets. This diversity in feeding ecologies is frequently made possible by microbes via horizontal gene transfer (HGT) and/or the establishment of mutualistic relationships. Herbivorous insects are often confronted with insufficient amino acids required for growth, as well as the need to break down the plant cell wall (PCW) material that is composed of fastidious polymers. Although insects ancestrally lack most PCW degrading enzymes (PCWDEs), our group has previously shown that leaf beetles (Coleoptera; Chrysomelidae) acquired PCWDE- encoding genes from fungi and bacteria

multiple times via HGT and that these acquisitions relate to the expansion of- and specialization in herbivory. In reed beetles (Coleoptera; Chrysomelidae; Donaciinae), we showed that this happened by replacing a horizontally acquired PCWDE – a pectinase – by a symbiont. However, multiple clade of reed beetles later lost the symbiont's pectinase after switching to a diet of pectin-poor plants. The reed beetle's symbiont also encodes for amino acid biosynthesis pathways, which support larval growth. This dual benefit – PCW digestion in adults and amino acid supplementation in larvae – would be inefficiently regulated via control of symbiont titres. Thus, given a symbiont's genome of 0.5 Mbp with only 4-5 transcription factors, we aimed to understand how these benefits can be regulated at a transcriptomic level throughout host life stages across four species. Indeed, the symbiont of the reed beetles specifically regulates benefits provided to the host. Furthermore, we show that reed beetle's own genome-encoded PCWDEs are upregulated during adult stages. As such, we show that expression of (ancestrally) microbe-provided benefits, both horizontally acquired and symbiont encoded, is tuned to the different ecological needs throughout the beetle's life cycle. This, together with a dynamic process of gene and/or symbiont acquisition, loss and replacement, makes microbes pivotal in both the expansion and constriction of insect ecological niches.

Crop diversification through intercropping shapes within-field soil bacterial community variation

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The current food system is in need of restructuring, to halt the environmental impacts associated with intensive monoculture such as chemical-overuse, biodiversity loss and soil degradation. Alternative agricultural methods, like intercropping, could increase sustainability by better utilizing the biological functions of the plant-soil ecosystem, particularly through the plant-associated microbiome. Soil microbes are a key player in supplying many ecosystem functions and contributing to plant performance. The effect of intercropping on the soil microbiome is a crucial but understudied part of integrating intercropping into the global food system. Here we characterize the effect of inter- versus intraspecific crop interactions on soil bacteria. We determined, through 16S rRNA gene amplicon sequencing, soil bacterial richness and community composition across 8 crop species and 16 crop combination in an organic strip-intercropping field. We find that overall, bacterial richness was not affected by crop identity or crop interaction type. In contrast, bacterial community composition was influenced by crop identity, with certain crops such as parsnip, potato and celeriac uniquely shaping their associated bacterial community in both intra- and interspecific crop interactions. We show that within one agricultural field, small changes in the soil environment brought on by unique soil-plant interactions can alter bacterial communities. Our study provides insights into the cascading effect of aboveground diversification on belowground diversification within one agricultural field.

sustainable agriculture, intercropping, soil bacteria, amplicon sequencing

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Plant-driven assembly of a soilborne phyllosphere disease-suppressive microbiome

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Plants can recruit disease-suppressive microbiomes in response to pathogen attack. For example, *Arabidopsis thaliana* plants infected above-ground with the obligate downy-mildew pathogen *Hyaloperonospora arabidopsidis* (Hpa) create a soilborne legacy (SBL) that increases resistance of subsequent plant populations growing in the same SBL soil. Moreover, leaves of distinct and geographically separated Hpa-cultures are dominated by near-isogenic Hpa-associated microbiota (HAM) that thrive on the infected plant and mediate the plant-pathogen interaction, increasing resistance. Here, we tested whether the HAM is recruited upon downy-mildew infection and inherited as SBL. We observed that plants inoculated with a gnotobiotic Hpa (gnoHpa) that is free of HAM establish SBL when grown in a natural soil, but not in a sterilized soil, indicating that SBL is the result of disease-induced microbiome recruitment by the infected plant. We showed that upon initial inoculation and consecutive passaging of gnoHpa over successive plant populations, specific ASVs accumulate in the phyllosphere that match HAM. From this we hypothesized that downy-mildew infected plants recruit disease-suppressive HAM from soil to their leaves. In this regard, we demonstrated that the phyllosphere is dominated by microbes that originate from soil but thrive in the aboveground, and that the HAM are such soilborne leaf-lovers. The HAM is effectively inherited by plants grown on SBL soil, colonizing the rhizosphere and root endosphere in low densities, but flourishing in the phyllosphere where they dominate the microbiome. Finally, we confirmed that passaging the phyllosphere microbiome of plants grown on SBL soil is sufficient to induce disease-suppression in a naïve plant population. Thus, HAM form a soilborne phyllosphere disease-suppressive microbiome that is recruited from natural soil upon downy-mildew infection and increase resistance when inherited by subsequent plant populations growing in the same soil.

disease-suppressive microbiomes, soilborne legacy, cry for help, recruitment, phyllosphere

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Session 5: Plant microbial community ecology

The genomic architecture of adaptation in nitrogen fixing symbionts

Joel Sachs (*Invited speaker*)

The root microbiome as a hotspot for horizontal gene transfer from bacteria to fungi

Arpan Kumar Basak, Thorsten Thiergart, Ram-Sevak Raja Kumar, Fantin Mesny; Stephane Hacquard

Horizontal gene transfer (HGT) is considered a major force in shaping prokaryotic genome evolution. However, the extent to which trans-kingdom DNA transfer from bacteria to eukaryotes has promoted functional diversification and adaptation to environment in eukaryotic genomes remains elusive.

Given that bacteria and fungi co-occur with their plant hosts since 450 MyA, we hypothesised that the multi-kingdom root microbiome represents a hotspot for HGT between bacteria and

fungi. We investigated the genomes of 120 fungal isolates representative of the root microbiome and identified > 700 orthogroups (OG) that were potentially acquired via trans-kingdom HGT. We found that HGT-derived OGs are highly distributed in Ascomycetes isolates and including *Plectosphaerella cucumerina*, a robust colonizer of *Arabidopsis thaliana* roots in nature. Interestingly we found a large fraction of these HGT derived OGs contribute to plant cell wall degradation and fungal metabolism.

Next, we hypothesised that *P. cucumerina* genes acquired from bacteria during HGT event might act as root colonization determinants conferring to fungal adaptation to its host. By combining fungal transcriptomics of infected roots and phylogenomic approaches, we identified list of candidate genes that are induced in *P. cucumerina* upon root colonisation and that unambiguously share bacterial ancestral origin. These genes encode plant cell wall degrading enzymes and effector-like proteins, that are reproducibly detected in the genomes of other *P. cucumerina* isolates that were isolated from diverse plant hosts across continents. Interestingly, these HGT derived genes are not conserved in *P. delsorboi*, and *P. plurivora*, suggesting that these genes emerged during the speciation event of *P. cucumerina*. Further, we aim to test the function of these candidate genes in root colonisation efficiency and fungal adaptation to its host.

Overall, we suggest that during evolution, genes responsible for fungal root colonisation have evolved from root-associated bacteria by inter-kingdom HGT.

Horizontal gene transfer, root microbiota, competitive advantage, microbe-microbe interactions, plant-fungi interactions

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Beyond defense: Glucosinolate structural diversity shapes recruitment of a metabolic network of leaf-associated bacteria

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Leaf bacteria are critical for plant health, but little is known about how plant traits control their recruitment. Aliphatic glucosinolates (GLSs) are a class of defense metabolites present in leaves of Brassicaceae plants in genotypically-defined mixtures. Upon plant cell damage, they are broken down into products that deter herbivory and inhibit pathogens. We studied the model *A. thaliana* genotype Col-0 which produces mainly 4-methylsulfinylbutyl-GLS and NG2, a genotype isolated from a local wild population, with mainly allyl-GLS. An aliphatic GLSs-free mutant is already available in Col-0 background and we generated one in NG2 background. Using bacterial growth analysis and 16S rRNA gene amplicon sequencing, we find that both GLSs differentially affect commensal leaf bacteria: In Col-0, GLS breakdown products are highly toxic to bacteria but have no effect on natural colonization of healthy leaves. In contrast, NG2 leaves enrich Burkholderiales bacteria, an effect also detected in nature. Indeed, intact allyl-GLS as a carbon source specifically enriched a Burkholderiales-containing community in-vitro. In this context, Burkholderiales depended on other bacteria that can metabolize GLSs. Metabolism of GLSs was accompanied by detoxification of the breakdown product with different efficiency depending on the GLS chemical structure. This might help to explain how GLS structural diversity influences bacterial recruitment in diverse genotypes. Though Burkholderiales did not utilize aliphatic GLSs directly, they increased community growth rates in-vitro. Together, these results suggest a broader role for well-studied defense metabolites in the plant microverse and may offer directions for targeted control of balance in the leaf microbiome.

Plant secondary metabolites; Glucosinolates; Phyllosphere microbiome; Community assembly; Burkholderiales

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Deciphering plant-rhizosphere interactions within a community framework

Sharon E. Zytynska (*Invited speaker*)

Understanding plant-rhizosphere interactions within a community framework is essential for advancing agricultural practices and ecosystem management. This talk will explore the rhizosphere microbiome as a dynamic community, emphasizing intricate interactions among microbes, other soil fauna, and aboveground species mediated by the host plant. Focusing on crop systems, I will highlight how these interactions can be exploited to enhance the use of microbial inoculants for promoting plant health and productivity. Environmental factors such as soil type, climate, and agricultural practices significantly shape rhizosphere interactions. I will discuss the resilience of rhizosphere microbiomes to environmental stressors and the supportive role of broader landscape factors like habitat diversity and connectivity. Additionally, the interplay between rhizosphere microbes, pest insects, and their natural enemies will be examined, showcasing integrated pest management strategies. Cultivating an integrated understanding of rhizosphere microbiomes, pest dynamics, and broader landscape factors is crucial for developing sustainable agricultural practices and enhancing crop resilience.

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Genome-scale metabolic networks predict rhizosphere microbiome suppressiveness against soil-borne pathogen *Ralstonia solanacearum*

Tianjie Yang, Xinrun Yang, Silvia Cretoiu, Gaofei Jiang, Yangchun Xu, Qirong Shen, Zhong Wei, Alexandre Jousset

The mechanisms involved in diversity-function relationships remains difficult to disentangle. However, the economically feasible whole genomic sequencing might provide a simple and reliable prediction of genomic traits with community function. Here we combined microbial genomics of model constructed communities with a biodiversity-ecosystem relationship framework and to examine if community resistance of invasion by a closely related plant pathogen can be predicted from resident community resource consumption patterns as inferred from genomic sequences based on the genome-scale metabolic network (GEMs). A series of microcosm experiments were conducted with engineered microbial communities representing a biodiversity gradient. We benchmarked our genome-based approach by comparing predictions based upon genome content to results obtained in a range of empirical biodiversity-invasibility functioning experiments performed in vitro and on host plant. We found that accuracy of calibrated GEMs based on resource use patterns of single bacteria reached to 90% on average. Predicted biodiversity-invasibility relationship generated from GEMs of rhizosphere microbial community showed significantly negative correlation. Furthermore, communities with a high inferred complementarity between species were shown to be more resistant to invasion. Specifically, predicted niche overlap and cross-feeding of rhizosphere microbial community calculated based on community GEMs could explain invasion resistance

in vitro and also on host plant. Our results demonstrate that functional metagenomics can be linked to theoretical community ecology to improve the prediction of microbial community function.

Genome-scale metabolic network, rhizosphere microbiome, resource competition, pathogen invasion

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Lessons from the wild: deciphering phyllosphere microbiome assembly and functions of wild green foxtail for designing synthetic communities

Xiaoyu Zai, Viviane Cordovez, Feng Zhu, Meicheng Zhao, Xianmin Diao, Fusuo Zhang, Chrats Melkonian, Marnix H. Medema, Jos M. Raaijmakers, Chunxu Song

Understanding microbiome assembly and functions of wild crop ancestors has been proposed as a novel strategy to ‘rewild’ crop microbiomes for enhanced growth and health. Here, we showed that biogeography, with variations in soil parameters and climatic conditions, and plant genetic distance significantly correlated with phyllosphere microbiome assembly of green foxtail, the wild ancestor of the cereal crop foxtail millet. Phyllosphere microbiome assembly was governed by stochastic and deterministic processes, with specific microbes emerging as core phyllosphere taxa based on higher relative abundance and prevalence across samples. Culture-dependent approaches enabled the isolation and identification of core bacterial and yeast community members and the design of synthetic communities (SynComs) that successfully suppressed infections of the domesticated foxtail millet by the foliar pathogen *Alternaria alternata*. This study provides unprecedented insights into the ecology and diversification of the phyllosphere microbiome of a wild crop ancestor across large geographic scales. We further show that this knowledge can be leveraged to design SynComs with beneficial traits for the health of the domesticated crop counterpart.

wild green foxtail; biogeography; phyllosphere; core microbiome; synthetic communities design

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Session 6: Microbiome-mediated effects on plant physiology and stress tolerance

Harnessing the power of deserts to make plants resistant to salt, heat and drought stress

Heribert Hirt (*Invited speaker*)

A major challenge for global food security is the lack of fresh water, making up only 3 % of total available water. 70 % of fresh water is currently used for agriculture but becomes increasingly under pressure by an increasing global population and industrialization. The effects of climate change enhance the frequency of heat and drought waves and scorge harvest yields by more than 60 % world-wide. At the same time, future agriculture is supposed to produce more food on less land and less inputs. We develop solutions by studying extreme ecosystems, where nature has selected solutions over thousands to millions of years. Our research on desert ecosystems provides effective solutions for smart agriculture. We identified specific desert

strains which can protect crops from heat or drought stress, can enhance plant water use efficiency as well as enhance crop salt stress tolerance. Consortia of desert microbes can induce stress tolerance to multiple stresses, significantly reduce irrigation requirements or partially substitute fresh with saline water. Importantly, desert microbe-treated crops show no penalty on growth or yield under non-stress conditions which is often seen in genetically engineered crops. These results show that plant-microbial symbiosis is a powerful tool for enhancing agricultural productivity providing an important contribution to human food safety.

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Microbial legacies drive plant health

Roeland L. Berendsen (*Invited speaker*)

Exploring Microbial Interaction for Plant Drought Resilience in *Phaseolus vulgaris* L.

Thierry A. Pellegrinetti¹, Ana V. R. da Silva¹, Izadora C. M. Cunha¹; Eduardo H. M. Boleta¹, Teresa M. L. Mafra¹, Lara A. Losovoi¹, Rodrigo Mendes², Siu M. Tsai¹, Lucas W. Mendes¹

Increasing global temperatures and diminishing water resources pose significant threats to food security. Exploring the role of the rhizosphere microbiome in improving plant drought tolerance presents a viable strategy to reduce crop losses and enhance stress resilience. The response of two cultivars of *Phaseolus vulgaris* L. to drought exposure was investigated, specifically exploring their distinct tolerance to drought. We hypothesize that the drought-tolerant (TL) genotype partially depends on the rhizosphere microbiome to manifest this trait, in contrast to the susceptible (SC) genotype. In a controlled drought stress experiment over 96 h (40% water field capacity), we observed that TL plants decreased their root mass while maintaining shoot mass stability, suggesting strategic resource allocation for drought tolerance. Additionally, prokaryotic community composition differed between TL and SC plants, with higher diversity observed in TL plants. Through network analysis and metabolic modeling, it was found that the TL genotype simplified its microbial networks under drought conditions, contrary to the SC genotype, which became more complex. This adaptation suggests a strategic shift in TL plants to modulate rhizosphere interactions, possibly reducing reliance on microbial cooperation by ensuring a steady supply of carbon through plant exudation. We mapped 17 metagenome-assembled genomes (MAGs) that exhibited differential abundance between TL (6) and SC (11), belonging to Acidimicrobia, Actinomycetia, Alphaproteobacteria, Bacteroidia, and Thermoleophilia classes. MAGs specifically associated with the TL genotype were enriched in genes linked to glycine-betaine production, halotolerance enzymes, sodium transport, and trehalose metabolism, suggesting a potential role in osmoprotection and desiccation relief. This implies that certain plant genotypes might recruit microbial taxa capable of enhancing drought tolerance through specific metabolic pathways. The findings illuminate the intricate rhizosphere microbiome dynamics in fostering plant drought resilience, offering a strategic approach to mitigate the effects of drought stress episodes and strengthen food production under climate change scenarios.

Rhizosphere microbiome, Drought tolerance mechanisms, Metagenome-Assembled Genomes

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Pseudomonas volatiles increase drought resilience through important transcriptional changes and by modifying the rhizosphere microbiome composition

Zulema Carracedo Lorenzo, Muhammad Rizaludin, Paolina Garbeva, Roland Berdaguer, Corné Pieterse, Christa Testerink, Marcel Dicke, Karen Kloth, Romyana Karlova.

Drought is an increasing problem for agriculture and a threat for food security worldwide. While the ability of beneficial soil bacteria to increase crop drought resilience has been shown, the underlying molecular mechanisms remain elusive. In this project we show that VOCs (volatile organic compounds) from three different *Pseudomonas* strains increase drought resilience in *Arabidopsis thaliana* and *Brassica oleracea*, in vitro and in vivo, respectively. By using RNA-sequencing and *Arabidopsis* mutants and reporter lines, we investigate the mechanism of drought alleviation. We showed that *Pseudomonas* VOCs induce changes in ABA-dependent and ABA-independent pathways, modulating sugar transport, and influencing secondary metabolite production important for plant-microbe interactions like glucosinolates and coumarins under drought stress, ultimately leading to enhanced drought tolerance in *A. thaliana*. Moreover, by performing amplicon sequencing of *Brassica oleracea* roots exposed to *Pseudomonas* VOCs, we identify a role of the *Pseudomonas* VOCs in changing the bacterial composition of the rhizosphere. Finally, we use GC-MS to identify the candidate *Pseudomonas* VOCs that could be responsible for the drought alleviation in the plants. This study provides valuable insights into the molecular and physiological mechanisms that explain the beneficial effects of *Pseudomonas* VOCs on enhancing crop resilience to drought.

Drought, *Pseudomonas*, VOCs, *Brassica oleracea*

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When in drought: causes and consequences of *Streptomyces* enrichment in plant roots during drought

Connor Fitzpatrick (*Invited speaker*)

Plants harbor diverse assemblages of microorganisms in epiphytic and endophytic habitats across above- and belowground organs. How these communities respond to and affect plant responses to abiotic and biotic stressors is a growing research focus. The enrichment of members of the bacterial genus *Streptomyces* in the plant microbiome under drought occurs across diverse plant species, exhibits high organ specificity, and is temporally stable. Yet the underlying factors driving this enrichment remain obscure. We use a combination of approaches to uncover the environmental, host plant, and bacterial mechanisms behind *Streptomyces* enrichment in plant roots during drought. In a common garden experiment with soil from locations spanning the climatic breadth in the continental USA we find that *Streptomyces* enrichment in roots during drought is highly prevalent yet its magnitude varies with several soil physical and chemical properties. Evidence from plant genetic and chemical manipulation support the hypothesis that dampened plant immunity under drought is necessary for *Streptomyces* enrichment. Finally, using cultured isolates we find that

Streptomyces enriched in roots during drought display unique behavior in response to different environments and possess a range of traits with potential importance for plant performance.

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Aboveground JA- and SA-pathways induce stress-specific root microbiome responses in *Brassica oleracea*

Marcela Aragón, Haris Spyridis, Michael Reichelt, Jonathan Gerschenzon, Marcel Dicke and Karen Kloth

Induction of plant defense responses aboveground has been linked to alterations in belowground root microbial communities, yet it remains unclear if these changes are stress-specific and beneficial for plants. In this study, we conducted a plant-soil feedback (PSF) experiment to explore whether root microbiome shifts are specific to the induction of Jasmonic Acid (JA) and Salicylic Acid (SA) phytohormonal defense signaling pathways. In the conditioning phase, we induced the JA and SA pathways in *Brassica oleracea* shoots by either infesting plants with insect herbivores or by simulating herbivory through the external application of phytohormones. Simulated herbivory consisted of treating plants with Methyl Jasmonate (MeJA) or SA. For infestation, plants were subjected to either *Mamestra brassicae* caterpillars as chewer insects or *Myzus persicae* aphids as phloem-feeding insects. From these plants, we analyzed the rhizosphere bacterial communities via amplicon sequencing, the phytohormone and glucosinolate profile of the shoots, and collected the conditioned soils to be used as soil inocula. In the feedback phase, we grew a second generation of *B. oleracea* plants in the six types of soil inocula obtained from the conditioning phase (JA-conditioned, SA-conditioned, and control-conditioned from both insect and simulated herbivory) and exposed plants to *M. brassicae*, *M. persicae*, or kept uninfested to assess resistance. Results confirmed that induction of JA and SA pathways was similar between simulated and real insect herbivory in the conditioning phase, as phytohormone and glucosinolate profiles were similar. Moreover, root microbial communities changed specifically according to the defense pathway induced. Differential abundance analysis revealed a decrease in the Proteobacteria in response to treatment with MeJA and chewing herbivory (JA-induced) but not SA and aphid herbivory (SA-induced), while ASVs from the Planctomycetota were enriched in both pathways, suggesting a general stress response on the roots. Despite changes in the root microbiome, there was no effect of soil inocula on plant growth or insect resistance during the feedback phase. Our study provides novel evidence that the induction of plant defenses alters root microbiome communities in a stress-specific manner.

Plant-soil feedback, root microbiome, JA pathway, SA pathway, caterpillar herbivory, aphid herbivory.

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Identifying disease-suppressive microbial consortia by dilution-to-extinction

Jiayi Jing, Adam Ossowicki, Vittorio Tracanna, Jos M. Raaijmakers, Paolina Garbeva, Marnix H. Medema

In disease-suppressive soils, root-associated microbiomes provide plant protection against infections by specific soil-borne plant pathogens. However, soil and rhizosphere microbiomes are highly complex, making it very challenging to pinpoint the microbial taxa and functions that underlie this phenotype. Previously, we screened 28 agricultural soils and found one soil (S11) that provides a consistent and high level of microbially mediated suppressiveness against *Fusarium culmorum* on wheat. To identify the microbial taxa and mechanisms involved in the *Fusarium*-suppressiveness of S11, a dilution-to-extinction approach was employed to perturb the microbiome composition and alter its disease-suppressive phenotype. Bioassays then indicated that the suppressive effect gradually decreased and was finally lost at increasingly higher degrees of dilution. Shotgun metagenomic sequencing of the rhizosphere microbiome along different dilution rates, and mapping of these reads to a whole-genome-sequenced collection of 353 taxonomically diverse microbial isolates from the S11 wheat rhizosphere, revealed changes in microbiome composition and functions. Siderophore-, type VI secretion-, and chitinase-related functions had a significant correlation to soil suppressiveness. Eleven bacterial strains were prioritized as potentially involved in suppressiveness based on correlation with the plant phenotype across samples. The 11 strains were introduced as a synthetic community (SynCom11) into the wheat rhizosphere in a gnotobiotic soil system and showed robust and significant suppression against *F. culmorum*. Modifications of the composition of this SynCom, i.e. deletion or replacement of specific members (i.e. *Pseudomonas*) diminished suppressiveness compared to SynCom11. Collectively, our study shows that microbiome perturbation by dilution-to-extinction is highly instrumental in disentangling the microbial taxa and functions of complex microbiome-associated plant phenotypes.

soil suppressiveness, dilution-to-extinction, synthetic community

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