

Book of Abstracts - Poster presentations

Session 1: Plant-microbiome interactions for sustainable agriculture

1. Wood boring beetles (*Neoplocaederus conradti* and *Idactus spinipennis*) as serious pest of *Boswellia papyrifera* tree in Ethiopia

Weldesebet Beze, Haimanot Teklemariam, Aklilu Nigussie, Fransco Vitali

Boswellia papyrifera is a species of flowering plant native to Ethiopia, which provides both wood and non-wood forest products, cultural, religious, economic and ecological significance. It is the main source of an aromatic resin known as frankincense from its bark, which widely used for local ceremonial consumption and as raw materials for international industries by exporting. It also creating wide job opportunities for many Ethiopians in frankincense tapping, collection, grading, processing, and marketing activities. Recently, the population of the tree is declining from time to time due to many factors including wood boring Cerambycidae, *Neoplocaederus conradti* Kolbe and *Idactus spinipennis* Gahan were identified as the serious pests cause of tree mortality in Humera district dry land areas, Ethiopia. The field surveys were assessed insect borer damage symptoms on improperly tapped, properly tapped and untapped boswellia stands by searching of holes, frass, and tunnel on the stem each observed trees. Improper boswellia trees are the most vulnerable for wood boring beetles. Field observations strongly suggest that proper tapping, rotational harvesting of frankincense and acceptable natural forest conservation crucial methods to prevent and minimize the impacts of wood boring insect on the survival of *Boswellia papyrifera* standing trees and its regeneration. This proper identification of wood boring beetles of the tree is an important foundation for future studies on effective and sustainable management of *Boswellia papyrifera* dry land natural forest in Ethiopia.

Boswellia papyrifera; cerambycidae; frankincense; wood boring beetles.

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2. Manipulation of the soil microbiome to enhance insect pest suppression in crop plants

Edward Cairns

Beneficial soil microbes can promote plant growth and induce plant resistance against insect pests. Combining multiple beneficial microbes into synthetic communities designed to simultaneously increase plant health and yield has a strong potential to support sustainable agriculture. My research aims to uncover the mechanisms behind these plant-microbe-insect interactions, seeking to optimise and boost plant health. Inoculation of barley roots with *Acidovorax radialis* and *Bacillus subtilis* shows aphid suppression in lab and field trials. Data from the field trial shows that plant rhizosphere microbial communities varied due to inoculation treatments, barley variety, and experimental year. *A. radialis* bacteria communicate using quorum sensing, and our experimental results using quorum-sensing mutants indicate that these molecules can modulate plant-bacteria interactions in barley. We use our system to explore the relative effects of direct bacteria-plant interactions compared to indirect interactions via the wider microbiome; with the aim to determine what key factors are needed

to develop synthetic crop microbial communities. Understanding how beneficial microbes interact with the plant and other microbes will enable the development of resilient microbial communities that offer protection across multiple crops and environments.

Soil microbiome, pest resistance, quorum sensing

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3. Ethylene signaling is essential for mycorrhiza-induced resistance against chewing herbivores in tomato

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"Root colonization by the mutualistic arbuscular mycorrhizal (AM) fungi can prime plant defenses aboveground, leading to Mycorrhiza-Induced Resistance (MIR) against different attackers, including insect herbivores. Still, the molecular plant-regulatory mechanisms involved in MIR remain widely unknown. Here, we show that the AM fungus *Funneliformis mosseae* protects tomato plants against two different chewing herbivores, *Spodoptera exigua* and *Manduca sexta*, and we explore the underlying molecular mechanisms.

To identify key regulatory pathways underlying MIR, we explored the differential plant response during the multiway interactions through a genome-wide transcriptional profiling of the leaves, followed by bioinformatics network analyses. Herbivore-triggered jasmonate (JA)-regulated defenses were primed in mycorrhizal plants, while differential regulation of ethylene (ET) signaling was observed, both at the basal level and further primed in response to herbivory. Ethylene is a complex regulator of plant responses to stress, and we hypothesized that fine-tuned ET signaling in mycorrhizal plants is required for the primed defensive response leading to MIR in mycorrhizal plant. We followed analytical, functional and genetic approaches to test this hypothesis and to get mechanistic insights on the ET signaling in MIR.

We found that ET biosynthesis and emission were higher in *F. mosseae* mycorrhizal plants, being further primed upon herbivory. Tomato lines deficient in ET synthesis or perception failed to display AM-primed JA biosynthesis and defense priming, and were unable to develop MIR against any of the herbivores. We demonstrate that ET signaling is essential for the primed JA biosynthesis and responses leading to MIR against herbivores in tomato."

Mycorrhiza-Induced Resistance (MIR), chewing herbivores, defense priming, hormonal crosstalk, ethylene, jasmonates

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4. Broad-range bacterial mutant screens in plant-associated *Sphingomonas* to identify key genes for successful and beneficial plant colonization

Ezgi Mehmetoglu Boz, Hugo Barajas, Fransizka Liesecke, Derek S. Lundberg

Isolates of the bacterial genus *Sphingomonas* abundantly colonize the leaves and roots of major crops, do not pose apparent harm to the plant and can provide plant growth benefits. Therefore, they may find agricultural applications as inoculants. Discovering the genetic basis of *Sphingomonas*' non-pathogenic plant colonization and its beneficial properties will make it

possible to identify natural bacterial strains with the most useful features. To pinpoint essential genes, we are thoroughly screening mutants, employing random mutagenesis across the *Sphingomonas* genome as well as targeted approaches. We have generated a pool of ~300,000 mutants for the model beneficial strain *Sphingomonas melonis* FR1, and we will apply the same strategy to several local Swedish *Sphingomonas* strains. In our mutagenesis process, we use an innovative method known as RB-TnSeq, which allows for identifying mutants in a population simply by amplicon sequencing of their unique barcodes. We are applying these mutant populations to in-vitro and in-planta conditions to identify differentially represented mutants across conditions. Those mutants missing in-planta may represent genes critical for in-planta success. Concurrently, we are using a CRISPR RNA-guided integrase-based reverse genetics system to disrupt specific genes that we believe may play an essential role in plant interactions or bacterial colonization, and also to introduce genetic barcodes for easily quantifying mutants and wild type strains by amplicon sequencing. The expected outcome will be genes required for *Sphingomonas* success.

Phyllosphere colonization, Plant beneficial bacteria, Random barcoded mutagenesis, Gene editing

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5. The Rhizosphere Microbiome Mediates the Suppressiveness of Spent Mushroom Substrate Amendment Against Cucumber Fusarium Wilt

Yuhang Qu, Zhenhe Su, Qinggang Guo

Fusarium wilt is a severe soil-borne disease caused by *Fusarium oxysporum*. Soil amendment is an effective approach to control soil-borne diseases. We demonstrate that soil amendment with 4% spent mushroom substrate (SMS) eradicates the incidence of *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) wilt in cucumber (CFW). This coincided with a 10-fold decrease of FOC in the cucumber rhizosphere amended with 4% SMS. Transplanting experiments highlight the pivotal role of the rhizosphere microbiome in controlling CFW and promoting cucumber growth. Further, 16S rDNA sequencing reveals a significant increase in beneficial bacteria, especially *Bacillus*, in the SMS-amended rhizosphere. Metagenomics analysis indicate that SMS amendment enhances key KEGG pathways such as ABC transport, two-component system and nitrogen metabolism in the cucumber rhizobacteria. Additionally, genes (*nirB*, *glnA*, and *gltD*) of rhizobacteria linked to dissimilatory nitrate reduction and glutamate biosynthesis increased following SMS application. *Bacillus* strains, SE58 and SE38, isolated from cucumber rhizosphere amended with SMS show 66% and 46% control efficiency against CFW respectively. Notably, the biocontrol efficiency of both SE58 and SE38 are enhanced in soil amended with 1% SMS, suggesting synergy between these *Bacillus* strains and SMS amendment. Taken together, this work shows that SMS protects cucumber against CFW, and that this is partially due to the recruitment to the cucumber rhizosphere of *Bacillus*. These results further our understanding of the protective effects of SMS and its role in the biological control of CFW.

cucumber Fusarium wilt, spent mushroom substrate, rhizosphere, microbiome, *Bacillus*
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6. Unveiling the influence of domestication on taxonomic and functional microbiome composition in foxtail millet

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Plant domestication has a significant impact on the taxonomic and functional composition of microbiomes, with hypothesized “missing microbes” playing a pivotal role in reinstating beneficial plant-microbe associations to enhance crop productivity. Foxtail millet (*Setaria italica*), domesticated from its wild ancestor green foxtail (*Setaria viridis*) over 16,000 years ago in China, presents an intriguing case study. However, the co-evolutionary trajectory and mechanisms through which foxtail millet domestication shapes root-associated microbiomes, and in particular the relationships of root exudates and developmental traits with microbiome composition, remain poorly understood.

In this study, we investigated the rhizosphere and endosphere bacterial and fungal communities of 16 cultivars, spanning multiple wild and domesticated stages, using amplicon sequencing (16S and ITS). Subsequently, through the integration of metagenomic and metabolomic analyses, we aim to decipher the intricate relationships among root phenotypes, root exudates and differential microbial functions.

Our recent findings highlight the intricate influence of domestication and plant genotypes on both rhizosphere and root endosphere microbiome compositions. Notably, we observed a greater impact on the bacterial and fungal communities within the root endosphere compared to the rhizosphere. Furthermore, domestication has led to the establishment of distinct root-microbiome relationships: wild cultivars with higher SRL (Specific Root Length) and smaller RTD (Root Tissue Density) exhibited significant associations with specific ASVs of *Devosia*, *Pseudomonas*, *Massilia*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and the fungal symbiont *Funneliformis*. Interestingly, these ASVs appear to be depleted from the root endosphere along the domestication trajectory. Conversely, domesticated cultivars with higher RTD and diameter displayed significant relationships with ASVs of the *Blii41*, which were enriched in the root endosphere of domesticated foxtail millet.

Collectively, these findings shed light on the relations underlying the co-evolutionary trajectory of foxtail millet and its microbiome during domestication.

Domestication, Foxtail millet, Root exudation, Rhizosphere and endosphere microbiomes, Microbial functions

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7. Boosting belowground organismic interactions for improvement of agroforestry systems performance

Cynthia Albracht, Melissa Reimann, Stephanie Werner

Agroforestry systems and biostimulants have been shown as valid option for agriculture in the face of climate change, providing a more sustainable and resilient setting to maintain food security in the long-term. In an effort to establish a better understanding of processes, aiming towards practical guidance for establishment and improvement of agroforestry systems the joint KlimAF project was initiated. Focusing on the optimization of alley cropping through

organismic interactions, we make use of well-established but also newly set up alley cropping systems across Germany consisting of poplar trees for short rotation, currant shrubs (*Ribes rubrum*) and a crop rotation. To investigate the impact of host-adapted biostimulants on plant performance, half of the lots received commercial mycorrhiza inoculants consisting of ectomycorrhizal and arbuscular mycorrhizal fungi. We aim to analyse the success of these additives for yield increase, general plant fitness and stress tolerance in alley cropping systems in dependency of local soil conditions, plant diversity within the system and management of the lots. Spanning several years with the KlimAF project, we are especially interested in a potential reservoir function of trees / shrubs for native and added mycorrhizal fungi, questioning whether inoculants successfully enhance and/or improve native microbial communities and stimulate soil amelioration. The KlimAF project aims to transfer any findings on optimization of agroforestry systems through organismic interactions into guidance for farmers who intend to set up alley cropping systems.

agroforestry, biostimulants, sustainable agriculture, mycorrhiza, alley cropping

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8. Responding to the cry-for-help: Exploiting disease suppressive microbes and signalling to control soil-borne crop disease

Caleb Morgan, Stephen Rolfe

Plant pathogens pose significant threats to global food security causing substantial yield losses. Conventional disease control methods, such as bactericides and fungicides, not only incur significant environmental and ecological costs, but are difficult to employ against soil-borne diseases. Microbial biocontrol offers a promising avenue to protect crops while minimising environmental impacts.

This project aims to further understand the beneficial relationships between plants and soil microbes. From the recruitment of beneficials to the mechanisms of microbial-mediated disease suppression, then translating this knowledge into practical solutions for field applications. Adopting a holistic approach, the project spans from field observations to laboratory investigations and back to the field.

Collaboration with farmers allows the identification of fields with disease pressure, particularly focusing on soil-borne diseases. Within these fields, we hypothesise that some individual plants will remain symptomless due to the development of a disease-suppressive rhizobiome. We will extract the rhizobiome of symptomless and diseased plants and test these in high-throughput assays for disease suppression. This process confirms whether the observed disease alleviation in the field is indeed microbially mediated. High-throughput sequencing of rhizosphere communities from healthy and diseased plants will aid in identifying potential disease-suppressive microbes, guiding isolation of strains of interest. These strains are then assayed for their disease-suppressing abilities, *in vitro* and *in vivo* to elucidate the suppressive mechanisms. Untargeted metabolomics will then be used to identify key metabolites which may be driving these interactions and suppressive mechanisms. As a means of translating findings back to the field, seed coatings will be developed using the acquired knowledge.

To expedite initial studies, soils previously identified as disease suppressive due to their microbial community are utilised in laboratory experiments to optimise approaches.

Translation, Agriculture, Metabolomics, Isolation

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9. Diversity of “yeast-like” within the seed microbiota

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Yeasts are the smallest unicellular eukaryotes of the fungi kingdom. This is a fungal lifestyle shared by several unrelated lineages distributed across the Ascomycete and Basidiomycete phyla. “Yeast-like” is used for the basidiomycete yeasts, for which the number of descriptions of new species has been the highest in the past 20 years. They have been identified and isolated mainly from the soil and phyllosphere compartments of temperate forests, cold and arctic climates. Metabarcoding analyses have highlighted their presence in the seed microbiota, however, to date, their role within this community is unknown. Based on the results of the meta-analysis by Simonin et al. (2022), an inventory of the diversity of “yeast-like” basidiomycetes from seeds will be presented. In parallel, with a high-throughput isolation method, a collection of more than 200 yeast isolates from five crop seeds (radish, bean, rapeseed, tomato and wheat) and three weeds (shepherd's purse capselle, common whitlow grass and hairy bittercress) has been obtained. The taxonomic identification of the isolates based on the sequencing of the ITS and the D1/D2 regions, revealed that they mainly belong to eight genera (Naganishia, Rhodotorula, Holtermanniella, Cystofilobasidium, Filobasidium, Sporobolomyces, Bullera and Vishniacozyma) with some that are highly plant-specific, while others are more ubiquitous (core taxa). These results represent a first step towards a better understanding of the seed-borne yeast community and their role for plant health.

Seed microbiota, yeast, Basidiomycete

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10. Exploring the Dynamics of Soil Resilience: Potential Associations between the Potato Rhizosphere and Nematode Infestation in Agricultural Soil

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"Plant parasitic nematodes are a prevalent and persistent problem in the cultivation of potatoes. Additionally, the soil microbiome plays an essential role in plant stress resilience, and microbes can be recruited in response to various stressors. The rhizosphere could therefore be considered as a supportive defense against nematode infestation. However, differences in soil microbiome dynamics between agricultural disease-suppressive soil (DSS) and nematode-infested soil (NIS) are still poorly understood. Therefore, exploring the dynamics of the soil microbiome and potato rhizosphere in a DSS and NIS was the focus of this study.

Two agricultural fields, one DSS and one NIS, were selected to grow potatoes in a controlled pot environment, with an additional group of inoculated NIS with DSS (IS). Soil composition, nematode analysis, and phenotyping were used to clarify soil characteristics and plant performance. Amplicon metagenomic sequencing of bacterial 16S rRNA and fungal ITS was

used to explore the dynamics of the microbiome composition throughout the growing season and along the rhizosphere-bulk soil gradient in the different soils.

The DSS and NIS showed contrasting dynamics in soil microbiome and rhizosphere formation. Bacterial diversity was higher in DSS, while fungal diversity was similar in both soils. In addition, the microbial composition of DSS had clear seasonal differences and a distinct bacterial rhizosphere establishment, while these dynamics were absent in NIS. Contrary, a distinct fungal rhizosphere was established in NIS but not in DSS. Furthermore, the IS microbiome composition was positively altered as diversity was increased and a bacterial rhizosphere was again able to establish. Shifts in abundance at the phylum level also highlighted the unique microbial compositions in each soil type. Altogether, the results suggest that each soil type harbors a different microbial composition with different dynamics during the season and in rhizosphere establishment, possibly related to a disease-suppressive capacity to nematode infestation."

Potato rhizosphere, plant parasitic nematodes, disease-suppressive soil, stress resilience, soil microbiome

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11. Short-term effect of high carbon amendments application on topsoil microbiome community composition during a growing season of winter wheat.

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In cropping systems where winter cereals follow N-rich crops like oilseed rape the risk of N losses in autumn is high. To reduce the N losses in these cropping systems implementation of high carbon amendments (HCA) is used. The potential of HCA to stimulate N immobilization via increment of microbial biomass N was demonstrated in lab incubation experiments (Reichel et al. 2018) and in long-term field trials (Zhao et al. 2017) already. Shifts in microbial community composition though HCA implementation have only been analysed in mesocosm experiments but not in field trials. We hypothesize that the changes in nutrient stoichiometry caused by the HCA implementation promotes the establishment of bacteria able to degrade complex C sources, such as Firmicutes and Acidobacteria. This leads to a release of less complex carbohydrates which could be used easily by copitrophic bacteria. A field-experiment in Dürnast (Germany) with a silty-loam soil was performed. Wheat straw was incorporated directly after harvest of oilseed rape in August 2022 and soil samples were collected from 0-10 cm after 6, 9, 11 and 12 months after implementation. We applied 16S rRNA gene metabarcoding, qPCR, microbial biomass analysis and determined grain yield of wheat. Results showed a slight increase in microbial biomass C in HCA plots, as well as an increase in alpha diversity. Grain yield decrease in HCA plots in comparison to the control. Most enriched taxa in HCA were detected in February and May, including genera described as cellulolytic, for example, *Niastella* and *Flavobacterium*. The proportion of copiotrophic bacteria was higher in the HCA treatment, which was especially prevalent in August 2023 after a long drought period in July. In summary, our data indicate that especially over winter the HCA application provides additional resources for the microbiome favouring microbial biomass in general and cellulolytic bacteria in particular.

high carbon amendment, soil microbiome, winter wheat, crop rotation

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12. Distinct changes in tomato-associated multi-kingdom microbiomes during *Meloidogyne incognita* parasitism

Enoch Narh Kudjordjie, Susana S Santos, Mette Vestergård

Background

The interplay between root-knot nematode (RKN) parasitism and the complex web of host-associated microbiota has been recognized as pivotal for the effective management of the pest. However, studies assessing this relationship have focussed on the bacterial and fungal communities, neglecting the unicellular eukaryotic members. Here, we employed amplicon sequencing analysis of the bacterial 16S rRNA, fungal ITS and eukaryotic 18S rRNA genes, and comprehensively examined how the microbiome composition, diversity and networking developed with time in the rhizospheres and roots of RKN-inoculated and non-inoculated tomato plants.

Results

As expected, infection with the RKN *Meloidogyne incognita* decreased plant growth parameters. At individual time points, we found distinct bacterial, fungal and eukaryote community structures in the RKN-inoculated and non-inoculated rhizosphere and roots, and RKN inoculation affected several taxa in the root-associated microbiome differentially. Correlation analysis revealed several bacterial and fungal and few protist taxa that correlated negatively with *M. incognita*. Moreover, network analysis using bacterial, fungal and eukaryotic data revealed more dynamic networks with higher robustness to disturbances in the RKN-inoculated than in the non-inoculated rhizosphere/roots. Hub taxa displayed a noticeable successional pattern that coincided with different phases of *M. incognita* parasitism. We found that fungal hubs had strong negative correlations with bacteria and eukaryotes, while positive correlations characterized hub members within individual kingdoms.

Conclusion

Our results reveal dynamic tomato-associated microbiomes that develop along different trajectories in plants suffering *M. incognita* infestation and non-infested plants. Overall, the results identify stronger associations between RKN and bacterial and fungal taxa than between eukaryotic taxa and RKN, suggesting that fungal and bacterial communities play a larger role in the regulation of RKN. The study identifies several putative RKN-antagonistic bacterial and fungal taxa and confirms the antagonistic potential previously identified in other taxa."

Plant parasitic nematodes, plant-associated microbiomes, tripartite networks, time-series analysis, correlation analysis

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13. Medicago crop wild relatives host a more multifaceted seed microbial community compared to domesticated lucerne of Australian origin

Shenali Herath, Jatinder Kaur, Timothy Sawbridge

The Australian dairy industry is being increasingly affected by challenging climate conditions. Hence, there is a necessity to create high quality forages that can perform well in future environmental stress conditions. Lucerne or alfalfa (*Medicago sativa* L) is a globally significant perennial pasture legume, crucial for livestock feed due to its high protein content, high digestibility and nitrogen-fixing ability. Recently, the seed microbes of crop plants are increasingly studied due to their potential implications in enhancing crop health and resilience. In this study, we examined the seed-associated bacterial microbiota of 18 lucerne and crop wild relatives (CWR) seed accessions. 16S sequencing has identified Gammaproteobacteria, Bacilli, Actinobacteria and Alphaproteobacteria as dominant bacterial classes across all *Medicago* accessions. Plant genotype has a profound effect on seed microbial assemblage, as assessed by RPCA analysis. Wild relatives have shown a more diverse microbiome than domesticated lucerne implying, domestication has led to a compositional shift in seed microbiota of cultivated lucerne compared to its CWRs. Various bacterial genera with potential plant growth promoting and biocontrol properties were isolated from the seeds. Average nucleotide identity-based (ANI) comparative genomic analysis revealed intra-species bacterial diversity within members of the *Medicago* seed microbiome and strains and 'genomovars' have significant genetic variability among closely related bacterial isolates. Small synthetic communities of three bacteria from two of the CWRs are currently being assessed for their potential influence on the long-term microbial composition of the lucerne microbiome. Our study has contributed to a deeper understanding of the microbial blueprint of *Medicago* seeds, providing novel insights into its' temporal dynamics and spatial organization. The discovery of CWR-specific bacterial endophytes could be addressed for optimizing seed quality and future breeding strategies aimed at fostering sustainable cultivation practices in forage agriculture

Lucerne, *Medicago*, Crop wild relatives, seed microbiome, plant-microbe interactions

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14. Effects of soil microbiome on crop quality and the impacts on gut health

Isabel Siles Asaff, Marieke Elfferich, Ciska Veen, Martijn Bezemer, Emilia Hannula

As the human population grows exponentially, global food security has been relying on the intensification of agriculture. These intensive agricultural practices focus on crop production quantity rather than its quality which has led to nutritional problems and consequently, increase in gut diseases across the globe. Moreover, land use intensification (LUI) has negative consequences on the environment leading to biodiversity loss, and specifically in soil it can negatively affect many soil functions. Soil microorganisms in agroecosystems have many benefits. They can improve soil structure, facilitate plant nutrient acquisition, induce plant resistance towards pathogens, and protect plants against abiotic stresses which can ultimately lead to better crop quality. Hence, it has been hypothesized that soil and human gut microbiomes are linked to each other through plant microbiome, thus suggesting that a

“healthy” soil microbiome can have benefits on human health. In contrast, soils harboring human pathogens could also be transferred to vegetable microbiomes leading to human health issues. Hence, the main objective of this project is to improve our understanding of plant-soil feedbacks to steer soil microbiomes for crop quality improvement by favoring taxa that are beneficial to plant health while repelling detrimental ones.

Land-use intensification, Plant-Soil Feedbacks, Soil Microbiome, Crop Quality, Human Health

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15. The mango microbiome as a source of novel biocontrol agents against fungal pathogens

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Mango malformation disease (MMD) is an economically important disease of mango (*Mangifera indica* L.) in both tropical and subtropical production areas. It poses a massive threat to mango industries worldwide. While *Fusarium mangiferae* has been reported as a main causal agent of MMD in mango-producing countries, the second emerging species is *Fusarium tuiense*, which is known to be predominant in Southern Spain and Brazil. The plant's health status is known to affect the structure of the plant microbiome greatly. The current research is the full microbiota study of mango plants, which includes both phyllosphere and soil analysis. The aim of this study is to compare the microbial communities of symptomatic and asymptomatic trees within MMD-affected area, using 16S and ITS amplicon sequencing, with the objective of identifying potential biomarkers associated with asymptomatic trees. The cultivable diversity of bacteria was analyzed as part of a larger study to select beneficial bacteria and utilize them as biocontrol agents to control MMD, as well as to enhance the productivity and sustainability of agroecosystems.

Mango Malformation, microbiome analysis, biocontrol, microbial isolation

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16. The impact of hedgerows versus neighboring land use on soil microbial communities

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Intensive agricultural practices profoundly affect aboveground and belowground microbiome diversity. The planting of hedgerows has been advocated as a way to increase aboveground diversity in agricultural landscapes, however, little attention has been given to how this plant system affects soil microbial communities. We expected the soil microbiome underneath hedges to differ from the adjacent agricultural land, because they receive different resource quality of litter, root exudates, and fertilizer. Therefore, we investigated how hedgerows along different types of land use impact the composition and diversity of bacterial and fungal communities in the soil. In the Maasheggen UNESCO Biosphere Reserve, three dominant land use types were selected: nature-conservation grasslands (NG), production grasslands (PG), and croplands (CL). For each land use, six fields were selected as independent replicates, and soil was collected from both the center of the fields and underneath two adjacent hedgerows. Soil DNA was extracted, and the bacterial and fungal communities were assessed by 16S and

ITS amplicon sequencing. Preliminary results reveal that bacterial communities beneath hedgerows have greater similarity to communities in nearby fields than those in hedgerows from fields with different management. With land use intensification (NG-PG-CL), the bacterial community composition changed (PERMANOVA: $R^2=0.276$; $p<0.001$), with an increase in Proteobacteria and Bacteroidota, and a decrease in Verrucomicrobiota and Actinobacteriota. By employing robust data science techniques such as random forest and co-occurrence network analysis, we will further analyze the data aiming to identify which groups act as biomarkers for each land use type and how the microbial communities relate to hedgerows and neighboring land use intensity. We conclude that while hedgerows matter for soil microbiome composition, the adjacent land use clearly influenced the structure and composition of the hedgerow soil microbiome.

Microbial ecology; Bacteria; Fungi; Land use; Biodiversity; Agricultural landscape

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17. Harnessing the complex microbiota of aquaponic systems for plant growth promotion

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Aquaponics is a soilless food production system that sustainably yields plant and fish biomass in a closed loop. It is based on water circulation and beneficial interaction between fish, bacteria, and plants. However, the nutrient flow reaching the plants often presents few available mineralized nutrients. Previous studies have highlighted the existence of a complex microbial network and the presence of potential plant growth-promoting (PGP) bacteria within aquaponic systems. One promising solution to improve nutrient cycling in aquaponics would be to harness such diversity by designing a PGP bacterial consortium. Following a high throughput sequencing (HTS)-guided approach, microbial communities were isolated from aquaponic lettuce (*Lactuca sativa* L.) rhizoplane and one of our aquaponic facility's sump and characterized through 16s rDNA amplicon sequencing. A literature-based pre-selection was done to limit the number of candidate bacteria, and a bottom-up approach was followed to design the PGP bacterial consortium. Six beneficial strains were selected based on their expressed PGP traits under in vitro conditions, including phosphate and potassium solubilization, indole-3-acetic acid, and siderophore production. The ability of the strains to live and grow in proximity to each other was assessed through an antagonism test. When used alone, some strains showed promising results by improving the growth of lettuce seedlings. Under aquaponic greenhouse and controlled conditions, lettuce biomass was significantly increased by applying one of the strains. The next steps will be to test the newly designed consortium by assessing its effects on seedlings' development and the lettuce growth cycle under aquaponic greenhouse conditions.

Aquaponics, Nutrient cycling, bacterial consortium, PGPB

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18. A molecular dissection of cryptic organophosphorus utilisation loci in plant

Bacteroidota

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In terrestrial and aquatic ecosystems, phosphorus (P) availability controls primary production, with consequences for climate regulation and global food security. Understanding the microbial controls on the global P cycle is a prerequisite for minimising our reliance on non-renewable phosphate rock reserves and reducing pollution associated with excessive P fertiliser use. Of particular importance is the role microbes play in remineralising plant-available phosphate from immobilised organic P complexes. The phylum Bacteroidota play important roles in complex carbon cycling, suppressing plant diseases, and transforming organic P into labile phosphate. This involves synthesising a unique phosphatase enzyme (PafA), which outperforms classical phosphatases in performing this desirable P-liberating process.

Initial protein biochemistry data revealed there are significant differences in enzyme kinetics between PafA forms from different representatives of the Bacteroidota phylum, with AlphaFold modelling suggesting gross changes in active site structure, despite sharing conserved catalytic residues. One distinct form encoded in the forest soil bacterium *Chitinophaga pinensis*, is found within a unique gene cluster enabling this bacterium to utilise phospholipid headgroups that other Bacteroidota, such as *Flavobacterium*, cannot. *Chitinophaga* spp. are abundant in natural soil ecosystems, and we propose they are key players in phospholipid recycling and increasing soil P availability. Proteomic analysis of the growth of *Chitinophaga* spp. on a variety of phospholipid substrates has enabled some initial characterisation of the phosphorus utilisation capabilities of these key microbes that could progress innovative approaches for enhancing sustainable agriculture.

Phosphatase, phospholipid, organophosphorus cycling

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19. Mini-granulated organic fertilizers composed from agro-food side flows impact soil microbial communities

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"Organic fertilization has been shown to have several benefits over mineral fertilizers. Firstly, organic fertilizers prevent runoff via slow release of the nutrients. Additionally, organic inputs enhance soil health by promoting microbial activity and improving soil structure. Interactions between plants and soil microbiome have been associated with plant vigour and growth promotion. Stimulating the right microbial life in the rhizosphere by applying certain inputs can lead to healthier root environments, in turn leading to more resilient plants. However, the effects of fertilizer types on soil microbial communities remains to be further elucidated, especially considering the wide array of possible inputs and their range of complexity.

DCM produces organic bio-fertilizers using a proprietary micro-granulation technology platform. The ultra fine organic granules can be tailor-fitted to specific applications with over thirty different raw materials available for blending, sourced from side flows of the food processing and agricultural industry. Examples are cacao shells, feather meal, blood meal, bone meal, vinasse and soy bean residues, among others. Each of these raw materials display unique nutrient-release-patterns. Each granule is homogeneously composed of the selected raw materials, allowing for fine and uniform distribution in the substrate, where the complex blend can act as food source for both the plant and the soil microbiome.

Through field experiments and laboratory analyses, we assessed the effects of diverse inputs, including chemical fertilizers, animal manures, and the specific DCM organic fertilizer granules, on soil microbial abundance, diversity, and activity. Results indicate that the fertilizer type significantly influences soil microbial life. Increased microbial biomass and activity were observed using the organic fertilizer granules. Moreover, shifts in microbial community composition were noted and extracted microbial communities displayed enhanced abilities to metabolize different carbon sources, indicating more functional diversity after application of the organic fertilizers."

organic, fertilizer, microbiome, agriculture

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20. Impact of soil biological activity on the physiological responses of soybean crops with the use of Microbiome replenisher

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The intensification of cultivation systems has impacted the balance of productive systems, affecting soil quality and plant productivity. The growing concern regarding these impacts has motivated the restoration of soil biological balance through the addition of a pool of microorganisms, through the use Microgeo[®] Technology - with the Process Continuous Liquid Composting - CLC. Thus, the microbiome replenisher is derived from a fermentation process (CLC) using bovine manure and/or rumen contents along with a carbon-based product that feeds microorganisms, causing them to multiply and produce secondary compounds. The products resulting from this process are metabolites of biological activity and a set of microorganisms that can stimulate soil microbial activity. The purpose of this study was to investigate the capacity of the microbiome replenisher to enhance soil quality, based on evaluations of biological parameters, as well as its influence on physiological activity and, consequently, on soybean grain yield in three regions: Lençóis Paulista – SP and two distinct areas in Primavera do Leste – MT. The trials consisted of two treatments: i) control and ii) Microgeo[®] (Microbiome replenisher), with 12 replications. Soil enzyme (acid phosphatase), photosynthetic parameters (leaf temperature, net photosynthetic, substomatal CO₂ concentration, stomatal conductance, water use efficiency and carboxylation efficiency) and production components were evaluated. The results of these studies demonstrate that the use of Microgeo[®]Technology proved to be efficient in enhancing acid phosphatase activity. In addition, the plants exhibited enhanced plant nutrition, resulting in noticeable improvements in photosynthesis synthesis and higher yields across all three locations. Given all this evidence, microbiome replenisher is a valuable tool for soybean producers aiming to maximize their yields and improve soil health.

Soil biocenosis; photosynthetic metabolism; soil-plant relationship.

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21. Impact of Amazonian Dark Earth on Mycorrhizal Communities in Degraded Pasture and Agricultural Soils

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Arbuscular Mycorrhizal Fungi (AMF) enhance nutrient acquisition and water uptake by plants, resulting in increased growth and crop production. These symbiotic relationships, driven by soil and plant characteristics, can result in changes in fungal community composition. In the Amazon region, *Urochloa brizantha* is the predominant pasture grass. This region is also home to a fertile and biodiverse soil known as the Amazonian Dark Earth (ADE), whose biotechnological potential remains unexplored. This study explored the effects of ADE inoculation (10%) on AMF communities associated with *U. brizantha* cv. Marandu in degraded pastures and agricultural soils. Rhizosphere soil was sampled, and DNA was extracted and sequenced using Oxford Nanopore Technology. Eleven genera and 70 species were identified from 310 OTUs according to the MaarjAM database. The genera *Claroideoglossum*, *Gigaspora*, and *Paraglomerace* were more abundant in agricultural soils, whereas *Archaeospora* and *Dentiscutata* were more abundant in degraded pasture soils, suggesting environmental preferences among different genera. Although ADE inoculation did not significantly affect richness, it increased diversity and altered community composition in agricultural soils. In degraded pasture soils, ADE inoculation reduced *Claroideoglossum* and *Klukospora* abundances and increased the relative abundance of *Glomus*, *Scutellospora*, and *Dentiscutatae*. In contrast, in agricultural soils, ADE inoculation decreased the relative abundance of *Diversispora* and *Glomus* and increased the abundance of *Acaulospora*, *Archaeospora*, *Gigaspora*, and *Scutellospora*. These results indicate that the inoculum source is not the main factor influencing the establishment of the new community, but the efficiency of the introduced genera to overcome the indigenous AMF community and its adaptability to the new environment. Finally, ADE inoculation can influence indigenous AMF communities differently depending on soil origin. Factors such as soil abiotic attributes, organism interactions, and host-fungi preferences are likely to drive these differences. Data analysis will be further employed to identify the main drivers of changes in the community.

Glomeromycota, interaction, fungi, nanopore sequencing, microbiome

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22. Microgeo Biotechnology applied in culture coffee

Maycon Leandro Soares, Patrick Vieira Silva

Studies on biological products are a challenge for modern coffee farming. On farms, coffee plants are affected by temperature, rainfall, relative humidity, agroecosystem, pest and disease management, and biological soil management, for example. Taking this into account, we aimed to evaluate microgeo biotechnology (microbiome replenisher through the Process Continuous Liquid Composting - CLC) on the physiological traits in coffee plants, as well as the technology on the physical, chemical and biological aspects of the soil, seeking to clarify the results obtained from the increase in productivity observed in three consecutive harvests. The study was carried out with *Coffea arabica* L. cv. Mundo Novo, established on 3.8 m x 0.70 m. The population was established in 2012, by using a mechanized system, with a drip supervision

system. The study farm is located in Monte Carmelo – MG, Brazil. Three comparative treatments were performed: (1) Control; (2) Microgeo® applied in soil; (3) Microgeo® applied in soil + plant leaf. The assessments were carried out during the 2020/2021, 2021/2022 and 2022/2023 harvests, which were defined according to weather and working conditions. During the period of plant development, the following were recorded: soil metagenomic analysis, soil enzymatic analysis, soil fighting dynamics, chlorophyll content, soil resistance to penetration, infiltration test and production. Microgeo biotechnology promotes a better interaction of communities of microorganisms in the soil. That worked in greater balance and suppression of pathogenic microorganisms, in addition to promoting improvements in the physical quality of the soil such as compaction and water infiltration and also promoting greater improvement in the physical quality of the soil such as compaction and water infiltration and also promoting greater rooting. Therefore, we concluded that Microgeo improves physical, chemical and biological quality in soil and plants, reflecting the significant increase in production in consecutive harvests.

Coffea arabica, regenerative coffee farming, microbiome

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23. Low land use intensity enhances soil fungal functional communities in arable lands

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Background and aims

Agricultural practices can influence soil microbial communities and their associated functions. However, the effects of agricultural practices on the functional groups of soil microbial communities remain poorly understood in the context of land use intensity. This study investigates how land use intensity influences soil microbial functional groups in arable lands.

Methods

We investigated geographically isolated arable lands embedded in a gradient of land use intensities in the Achterhoek region, the Netherlands. We analyzed bacterial and fungal community composition using high-throughput amplicon sequencing. Additionally, we mapped ecologically relevant putative functional groups of identified bacteria and fungi based on FAPROTAX and FungalTraits.

Results

The results reveal that increases in agricultural land use intensity coincide with a significant decline in the diversity and composition of many soil fungal functional groups, while marginal effects were observed for functional groups of the bacteria. Specifically, irrigation and pest control emerged as the two main practices influencing the community assembly of saprotrophic fungal functional groups. Moreover, the connectivity between soil fungal functional groups decreased under higher land use intensity, which could have important consequence for belowground interactions.

Conclusions

Our findings indicate that low land use intensity has positive impacts on the diversity of soil fungal functional groups. This can potentially sustain associated ecosystem functions and consequently promote sustainability in agroecosystems.

agroecosystems, agricultural management practices, bacteria, fungi, soil microbial functional groups

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24. No Fear of the Dark: Exploring Microbial Diversity of Amazonian Dark Earths to Enhance Plant Growth

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Amazonian Dark Earths (ADE) are nutrient-rich soils from the Amazon rainforest that harbor a vast microbial diversity, supporting plant growth and pathogen protection. Despite their importance, most of these microorganisms remain unidentified or poorly described until the present. In this study, we used culture-dependent and independent techniques aiming to explore the “black box” of ADE and identify microorganisms with biotechnological potential. ADE samples were collected from a secondary forest in Iranduba, Amazonas, Brazil, at five points in the arable soil layer (0-20cm). Metagenomes were sequenced using an Illumina NovaSeq 6000, and metagenome-assembled genomes (MAGs) were generated using MetaSPAdes, MetaBAT 2, CONCOCT2, and MaxBin2 in the KBase Platform. The genomes were annotated using the GTDB database. We obtained 36 good-quality genomes (>50% completeness, <10% contamination), including plant-growth-promoting bacteria (e.g., orders Rhizobiales and Burkholderiales), nitrogen fixers (e.g., family Nitrospiraceae), and ammonia-oxidizing archaea (e.g., family Nitrososphaeraceae). Additionally, we isolated microorganisms on four culture media (King’s Agar B, Bushnell-Haas, R2A, and Luria-Bertani) at three different temperatures (26, 55, and 65 °C). Sanger sequencing of 128 isolates revealed a predominance of the order Bacillaceae. We also found species of *Streptomyces*, *Burkholderia*, and *Lysinibacillus*, all taxa related to soil quality and plant growth promotion. Our results shed light on the microbial diversity of Amazonian Dark Earths and highlight its potential applications in promoting plant growth, among other contexts. Moving forward, our research will focus on evaluating the capabilities of these microbial isolates, including their effectiveness against pathogens, production of phytohormones, and ability to solubilize nutrients. By delving further into the microbial diversity of ADE, we aim to uncover solutions that benefit both plants and soils.

Amazon rainforest, Biotechnology, Culturomics, Next Generation Sequencing, Soil Health

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25. Rhizosphere engineering by microbiome transplantation for salinity stress mitigation in *Solanum lycopersicum*

Salila Pradhan, Shubham Dubey, Shilpi Sharma

Rhizosphere, the narrow region of soil that is directly influenced by plant roots, is rich in microbial diversity with complex plant-microbe interactions. Strategic modification of the rhizosphere microbiome helps in enhancing plant fitness and mitigate stresses encountered by plants. The objective of the study was to mitigate salinity stress in salt-susceptible tomato plant by a microbiome-based top-down rhizosphere engineering approach involving acclimatization of the microbiome to salt stress over successive plant growth cycles (PGCs). Rhizosphere microbiome transplantation was performed by preparing the inoculum from the rhizosphere microbiome of the phenotypically best performing plant under salinity stress and applying it to the subsequent PGCs with ramping up of salt concentration at alternate cycles. The passaging of microbiome inoculum has been done for twenty PGCs till now. The outcome of this study demonstrated that rhizosphere microbiome acclimatized across successive passages, and enhanced plant tolerance to salinity stress. There was an increment in shoot length, root length, dry weight, membrane stability index, and chlorophyll and carotenoid contents in plants in when inoculated with the acclimatized microbiome. Microbiome inoculation also gradually decreased the levels of stress indicators like proline and malondialdehyde compared to salt-treated plants without inoculum. There was a significant difference in root architecture system between salt-treated plants, with and without microbiome inoculation. The expression of salt stress responsive genes were also studied to analyse salt tolerance level in roots of tomato plants. The microbial shift along the acclimatization process was also characterised by amplicon sequencing of 16S rRNA gene and ITS marker. This study emphasizes the effectiveness of the multi-passaging technique for microbiome acclimatization as a promising and sustainable strategy to mitigate the impact of salinity stress in plants.

Rhizosphere engineering, microbiome transplantation, salinity stress, *Solanum lycopersicum*, stress marker analysis, microbial diversity

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26. Root-knot nematode modulates the susceptibility of plant rhizosphere bacteriome to pathogen infection

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The rhizosphere microbiome of plants plays an important role in resisting pathogen invasion. Complex environmental conditions in soil often result in the invasion of collaboration of multiple pathogens or pests such as nematodes, through decreasing plant resistance. Yet, it is still unknown how plant parasite nematodes and pathogens achieve composite infection by affecting the rhizosphere microbial community. Here, we conducted a series of inoculation experiments with root-knot nematodes and *Ralstonia solanacearum* to explore how root-knot nematodes help *Ralstonia solanacearum* invasion into plants by altering the function of rhizosphere microbiome. Our results found that invasion of root-knot nematodes affected rhizosphere microbiome composition by mediating the expression of plant genes, stimulating an increase in sugar substances such as fructose, galactose and reducing lycopene. In subsequent *in vitro* and *in vivo* experiments, we found that changes in plant root metabolites reduced the abundance of anti-pathogen bacteria such as *Bacillus* while facilitating the abundance of *Ralstonia solanacearum*. Moreover, we found through the construction of synthetic communities that the altered metabolic composition of the root system can alter the composition, transforming the root microbial community from inhibitory to susceptible,

promoting the growth of pathogens in the soil and ultimately leading to plant disease. Overall, our research sheds light on the complex interplay between parasite nematodes, bacterial pathogens, and the rhizosphere microbiome in influencing plant health. Understanding these dynamics is crucial for developing strategies to mitigate the impact of multiple agricultural diseases.

Root-knot nematode, rhizosphere, *Ralstonia solanacearum*, plant metabolites

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27. Mixed Synthetic Microbiome "Bacteria - Filamentous Fungi - Yeasts" for Protecting Radish Seeds Against the Phytopathogenic Agent *Alternaria brassicicola*.

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The microbiome is an integral part of plants and plays a major role in their health. In recent years, the concept of synthetic microbial communities (SynComs) for plant protection has emerged and appears to be a promising alternative to phytopharmaceutical products. Current studies use in majority the bacterial microbial communities, but it seems that SynComs inspired by the natural microbiome could be more effective to protect plant from biotic and abiotic stress.

We designed and tested SynComs inspired by natural microbiome of radish seeds in the context of protection them from phytopathogenic fungus *Alternaria brassicicola* (Abra43). In order to reduce the symptoms caused by Abra43 on radish seedlings, 7 SynComs composed of one to three different groups of microorganisms (bacteria, filamentous fungi, and yeasts) were tested.

Our study demonstrated the ability to manipulate the bacterial and fungal fractions of the radish seedling microbiome through the inoculation of SynComs. Interestingly, we observed that the most effective SynCom for reducing symptoms and the relative abundance of Abra43 was the most diverse (BFY), followed by other SynComs including filamentous fungi (BF and F). These results suggest the predominant role of filamentous fungi in the defense of the radish seedlings against Abra43, but these effects are enhanced in the presence of bacteria and yeasts. We identified the most effective microorganisms for limiting the colonization and transmission of Abra43 as *Alternaria section alternata*, as well as *Plectosphaerella*, *Cladosporium*, *Acremonium* and the bacteria *Pseudomonas fluorescens*, *marginalis* and *viridiflava* and *Pantoea agglomerans*.

These promising results provide proof of concept for the use of mixed SynComs on seeds to limit the impact of a seed-transmitted phytopathogenic agents. This approach allows us to modulate the seedling microbiome assembly through seed inoculation and could limit the establishment of phytopathogenic agent and obtain healthy seed lots.

radish, *Alternaria brassicicola*, seedlings, seeds, seed microbiome, synthetic microbial communities

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28. Symbiotic Futures: Leveraging Seed Microbiomes for Sustainable Cannabis Cultivation

Carolina Lobato, João Machado de Freitas, Daniel Habich, Isabella Kögl, Katherine Cuadros-Patiño, Gabriele Berg, Tomislav Cernava

The germination of seeds initiates a plant's journey towards resilience and adaptability to disease, nutrient scarcity, competitive pressures, and environmental stressors. Beyond being mere repositories of plant DNA, seeds host intricate microbial communities whose transmission across successive plant generations can significantly influence the ecological and evolutionary dynamics of plant symbiosis. However, plant domestication often led to a considerable reduction in the genetic diversity of cultivated plants with potential repercussions in genes involved in plant-microbiome interactions. The extent to which breeding can impact the seed microbiome is unknown. In our study, we conducted an extensive amplicon sequencing analysis of the bacterial seed microbiome of 46 different *Cannabis sativa* L. genotypes and hypothesized that the heterogeneous genetic background of *Cannabis* reflects on its seed microbiome. We detected higher bacterial diversity in less domesticated genotypes and a genotype-specific microbiome mainly composed of Gammaproteobacteria, Bacilli, Actinobacteria and Alphaproteobacteria and Actinobacteria. Further, we identified the bacterial biomarkers from less domesticated genotypes and tested their potential benefits on *Cannabis sativa* L. plants. From those, *Peribacillus frigitolerans* C1141 showed significant effects on plant growth under field conditions. Whole genome sequencing (WGS) analysis on *Peribacillus frigitolerans* C1141 showed the presence of important plant growth-promoting trait genes, including ones with direct effects on the plant such as biofertilization, bioremediation and phytohormone production. This study highlights the potential of seed microorganisms in providing biotechnological solutions for sustainable agriculture and the importance of preserving seed microbiomes in plant breeding.

plant microbiome, seed endophytes, plant breeding, plant-microbe interactions, bacterial communities

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29. Predicting germination and soil seed bank persistence of agricultural weed *Alopecurus myosuroides* from soil and seed microbiomes

Jonathan Binder, Thomas Holloway, Ben Oyserman, Kazumi Nakabayashi, Gerhard Leubner-Metzger

While often disregarded and not as well understood as microbe-root or microbe-shoot interactions, microbe-seed interactions can have important impacts on plant health. Weed seed persistence in the soil seed bank depends on dormancy, longevity and defence as key traits. Although it is generally understood that soil microorganisms contribute to weed seed decay, clear and strong relationships and mechanisms are largely unknown. It is posited that certain soil management practices increase the rate of weed seed decay by microbial activity, but neither specific microbial species nor practical strategies have been rigorously studied or confirmed. While there is some research on soil microbial effects on weed seeds, few (if any) studies have investigated how innate seed microbiomes may modulate persistence in the soil seed bank.

To gain insight into the microbe-seed interactions of agricultural weed *Alopecurus myosuroides* (“blackgrass” in the UK) and their implications for management and control, we studied the

effect of tillage, an important agricultural tool to control weeds and a driver of soil microbiome assembly, on *A. myosuroides* germination in a controlled lab setting with soils collected from different fields. Results of how different tillage regimes affect the dynamic microbial communities of seeds and soil obtained by 16S and ITS amplicon sequencing will be presented. Investigation into how soil C content modulated microbe activity and, by extension, seed germination patterns will also be discussed. Our experimental work in collaboration with Syngenta (UK, USA, Switzerland) as industrial partner aims to deliver an improved understanding of how soil and seed microbiomes are formed and their potential to affect weed seed persistence in distinct agricultural practices. A model, based on observations, to predict seed decay and germination and, by extension, improve weed management will be proposed.

weed science, seed, amplicon sequencing

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30. Microbial ecology in soilless cultivation

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Soilless systems are common in greenhouse horticulture, where crops and ornamentals are grown on water-based hydroponics, inert material as rockwool or organic potting soil mixes. The advantage of these systems is that they use less land for crop production than agriculture. They also have the potential for less environmental pollution and have the potential to use less water if water is recirculated. A symbiotic microbiome is important for crop health and disease suppression in soilless systems. However, many soilless systems have a low microbial diversity, at the start of cultivation, compared to soils as they are thoroughly hygienised and lack a history with plants grown in these systems. Thus, soilless systems may lack a beneficial microbiome that increases resilience against pathogens and improves the availability of nutrients for the crop. Furthermore, most knowledge on the role of microbes in cropping system resilience against pathogens or the breakdown of organic material to improve plant nutrition comes from agricultural soils, which may not resemble the conditions in soilless systems.

Here we discuss future research needs to optimize microbial functioning and microbe-plant interactions to improve crop production and ornamental plant growth in horticulture systems. Plant-microbiome interactions in soilless cultivation are influenced by the abiotic factors, the type of soilless system used and the quality of the water and organic materials in the nutrient solutions. These elements function as one and not separately from each other and are important for future growing systems.

soilless, horticulture, microbiome, pathogen suppression, biocontrol

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31. Catching cereal killers: uncovering the functional potential of phyllosphere yeasts to control a mycotoxigenic pathogen

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Despite the increasing recognition of the importance of the leaf-associated (i.e. phyllosphere) microbiomes for plant growth and health, the taxonomic and functional diversity of phyllosphere microorganisms is still largely unknown. Yeasts are versatile microorganisms that are abundant on leaf surfaces and inside leaf tissues. Due to their wide metabolic diversity, they can withstand several stressful conditions (e.g. fluctuating nutrient and water availability, temperature, UV radiation) and compete with plant pathogens for nutrients and space in the phyllosphere. Ongoing research in my group found that specific yeasts not only inhibit the growth of the mycotoxigenic fungus *Fusarium graminearum* but also suppress mycotoxin biosynthesis in vitro and in planta. Contamination of cereal crops by mycotoxins from plant pathogenic fungi leads to vast economic losses and poses a serious threat to food and feed safety worldwide. Leveraging yeasts to influence the proliferation of pathogens, in particular mycotoxigenic fungi, provides novel opportunities for sustainable crop production and food safety. In this talk, I will present the several 'omics approaches we are employing to decode the functional potential of phyllosphere yeasts and the specific chemical and molecular mechanisms underlying the suppression of mycotoxin biosynthesis in plant pathogenic fungi.

yeasts, phyllosphere microbiome, (a)biotic stresses, microbial interactions, mycotoxin

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32. Back to the roots: impact of rhizobacteria on the life-cycle of the root parasite *Striga hermonthica*

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**Shared first authors*

One of the major bottlenecks to control the root parasitic weed *Striga* is its complex life cycle. Among the current management practices, resistance breeding, intercropping, and push-pull have been implemented in several sub-Saharan African countries where *Striga* causes major yield losses of sorghum, millet, maize, and upland rice. To date, however, none of these strategies are singularly

effective. We discovered that specific soil and root-associated bacteria can i) disrupt the early stages of the parasite's life cycle through the production of volatile organic compounds, and/or

ii) degrade host-derived germination signals and haustorium-inducing factors. To increase the success rate of microbe-mediated control strategies across a diverse range of agroecosystems, we first determined the core rhizobacterial community of sorghum. Experiments with three microbiologically distinct Ethiopian field soils and twelve (local) sorghum genotypes revealed that taxa representative of the bacterial genera *Pseudomonas*, *Rubrobacter*, and *Streptomyces* comprised the core bacterial community. Several *Pseudomonas* isolates exhibited volatile-mediated suppressive effects on *Striga* seed germination ranging from 36-100% inhibition relative to non-exposed *Striga* seeds. GC-based detection and identification of the volatiles produced by the *Pseudomonas* isolates revealed significant negative correlations with *Striga* seed germination for the volatiles S-methyl methanoethiosulphonate (SMMTS) and dimethylsulfide (DMS). Subsequent greenhouse bioassays showed a 10 to 60% reduction in *Striga* attachment when sorghum is inoculated with some of these isolates when compared to the control. In subsequent experiments, we further showed that *Arthrobacter* and *Microbacterium* isolates can significantly reduce the levels of the germination stimulant orobanchol and the haustorium-inducing factor syringic acid in sorghum root exudate leading to less *Striga* seed germination and haustorium formation. In collaboration with local research institutes, the suppressive bacterial isolates will be tested for *Striga* control in on-farm trials in sub-Saharan Africa.

core rhizosphere microbiome, Sorghum, *Striga*, rhizobacteria mediated suppression, haustorium inducing factors

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33. AVOCADO RHIZOSPHERE AND PHYLLOSHERE AS A SOURCE OF BACTERIA FOR THE BIOCONTROL OF AVOCADO FUNGAL DISEASES

Marcos Pedraza Rubio, Mariia Kolodeznaia, Victor J. Carrion, Francisco M. Cazorla

Plants associate with a complex array of microorganisms in both the rhizosphere (soil surrounding the root and influenced by its exudates) and phyllosphere (aboveground part of terrestrial plants). These microorganisms in close contact with the plant can significantly impact plant health and development, so plants can recruit beneficial microbes to enhance their ability to defend against pathogens. In this sense, avocado popularity has arisen in Spain in recent decades, thus being the leading avocado producer in Europe. Avocado crops are facing a reduction in its production due to fungal diseases like white root rot (caused by *Rosellinia necatrix*) and dieback of branches (caused by members of the *Botryosphaeria* group).

This study aims to analyze avocado plant biodiversity and microbial populations to identify bacteria associated with healthy avocado trees and assess their biocontrol potential against those fungal pathogens. After 16S and ITS rRNA sequencing, differential abundance analysis revealed the bacterial taxa associated with healthy and diseased avocado trees. It was reported the enrichment of bacteria in healthy trees, such as those from the genus *Bacillus* and *Flavobacterium*. This study highlights that the avocado microbiome rhizosphere and phyllosphere are a rich source of bacteria with antagonism against fungal pathogens of high economic relevance. These bacteria could be used as effective biological control agents in agriculture, offering a more sustainable approach to combating fungal diseases.

Microbiome, phyllosphere, rhizosphere, avocado, fungi, biocontrol

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34. Niche competition by biotrophic smut fungi: Ustilago-induced host resistance to secondary infection

Tiwari R, Werner J, Zuo W and Doehlemann G

The smut pathogen *Ustilago hordei* belongs to the group of Ustilaginales, members of which infect many economically important crops, including maize, wheat, barley, oat, and sugar cane. Smut fungi are biotrophic pathogens that depend on living hosts to proliferate. Hence, they need to avoid opportunistic infection of their host. The current study explores, if smuts can actively protect colonized plant parts from secondary infection by other pathogens.

At first, we tested the susceptibility of *Ustilago*-infected host leaves to other pathogenic fungi. We found that maize leaves colonized by *U. maydis* exhibit a strong resistance against the necrotrophic fungal pathogen *Botrytis cinerea*. The similar effect was seen in barley leaves colonized by *U. hordei*, where infection by *B. cinerea* was significantly reduced. Microscopic analysis and fungal biomass quantification of *B. cinerea* development in *U. hordei* colonized leaves was done to pinpoint, at which stage the secondary infection is stopped.

Next, we asked if effectors of *U. hordei* are involved in microbiota manipulation. For this, we performed in-vitro induction of effector genes expression and found that this leads to distinct antimicrobial activities of *U. hordei* towards barley-associated bacteria. *U. hordei* co-incubated with barley associated bacteria showed differential regulation of effector genes. In future, we will investigate, to which extent microbial antagonisms of *U. hordei* result directly from antimicrobial activity of proteinaceous effectors, or indirectly from the modulation of host defense pathways.

Antimicrobial activities, Antagonisms, Effector, host defense, In-vitro induction, Microbiota, Smut fungi

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35. Extraction of lignin from solid coffee waste and its biotransformation

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The versatility of plant biomass derived from the coffee industry is remarkable, as it serves various purposes in the cosmetic, food, and pharmaceutical industries. Lignin extraction, for instance, presents itself as very promising since it can be used for industrial materials, antioxidants, adsorbents for heavy metals, anticancer agents, and dyes. Deep eutectic solvents aid in separating lignin from other biomass components, making extraction more efficient and sustainable. Biotransformation emerges as a promising approach to manage coffee biomass residues, with endophytic fungi and actinomycetes as good biocatalysts in this type of reaction. These microorganisms produce enzymes that can catalyze the conversion of biomass components. In this sense, we evaluated some experimental conditions to extract the lignin and cellulosic material from solid coffee waste samples. The extracted material was characterized using classical techniques such as FTIR (Fourier Transform Infrared

Spectroscopy) and TGA (Thermogravimetric Analysis). Following the extraction, the lignin was used in biotransformation reactions, using fungal and bacterial strains as biocatalysts. Preliminary results have shown that it was possible to extract lignin from the coffee waste biomass. Biotransformation assays with pre-treated biomass using deep eutectic solvents revealed enzymatic activity of laccase and lignin peroxidase, highlighting their importance in producing valuable compounds from biomass.

actinomycetes, deep eutectic solvents, biotransformation, lignin, biocatalysts, coffee

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36. Exploring the Impact of Potassium Fertilizers on Soil Microbiome in Soybean Crops: Insights for Sustainable Agriculture

Alice Ferreira Alves, Amanda Manuely da Silva Oliveira, Felipe Martins do Rêgo Barros, Fernando Dini Andreote

Effective soil management is paramount for agricultural systems, with fertilization practices playing a crucial role in boosting production. However, understanding their interaction with other production components remains limited. Fertilization sustains soil fertility and amplifies crop productivity by enhancing plant nutrition, rendering it indispensable for agriculture. Addressing the escalating demand for agricultural productivity stands as a pivotal challenge of the 21st century, necessitating exploration of solutions for ensuring food security. Despite this urgency, scant attention has been given to understanding how soil microbiota responds to different potassium (K) fertilizers. Hence, this study aimed to scrutinize acid phosphatase enzyme activity in soybean rhizospheric soil under varied potassium fertilizer applications. The greenhouse experiment employed agricultural soil with standard fertilization (100 kg/ha-1 adapted for g/pot) using potassium sources with differing solubility rates: potassium chloride (KCl), potassium sulfate (K₂SO₄), and phonolite rock powder, each with 3 repetitions. Findings revealed that KCl and K₂SO₄ application in V3 favored Actinobacteria, Verrucomicrobiota, Planctomyceota, and Acidobacteriota. Similarly, the control mirrored KCl, while bulk soil displayed lower Acidobacteria and Planctomycetota abundance. Notably, in R1, KCl and K₂SO₄ treatments exhibited Cyanobacteria abundance differences. Furthermore, phonolite rock powder application led to lower richness compared to KCl, aligning with taxonomic composition in both phenological stages. Correlation analyses unveiled that microbial community abundance was independent of soil enzymes, suggesting no significant influence. Thus, enzymatic variables failed to exert a significant effect on the bacterial community concerning the treatments. These findings offer valuable insights into rhizosphere microbiome responses to various potassium fertilizer applications, be it chemical or alternative sources like rock powder. They also shed light on rhizosphere diversity and the potential presence of functional redundancy in alternative fertilizer applications.

crop productivity; rhizospheric soil; soybean crops; microbial communities

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37. Differences among fungal microbiomes associated with tar spot of corn in Ecuador, Guatemala, and Indiana

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Tar spot of corn is a recently emerged disease in the USA and Canada, with a broad geographic distribution in Central, South, and North America. This disease is caused by the obligate pathogen *Phyllachora maydis* and is associated with *Monographella maydis* (syn. *Microdochium maydis*) and *Coniothyrium phyllachorae* in Mexico, but not in the United States. *M. maydis* was initially considered to cause fish-eye lesions and *C. phyllachorae* was described as a mycoparasite of *P. maydis*, but their presence and roles in tar spot disease are still unclear.

Fungal microbiomes associated with tar spot lesions can be influenced by multiple factors such as geographic location, environmental conditions, and corn cultivar. To test the hypothesis that fungal communities present in tar spot lesions differ by location, we collected tar spot-diseased leaves in Indiana, Guatemala, and three regions in Ecuador with different environmental conditions. A secondary goal was to test whether *Coniothyrium* and *Monographella* are present in locations with different environmental conditions than Indiana, where they so far have not always been detected.

The alpha and beta diversity analyses showed a clear distinction between fungal microbiomes in Ecuador, Guatemala, and Indiana; samples from the same location clustered together. The most abundant taxa in the three countries are *P. maydis*, *Paraphaeosphaeria*, and *Sordariomycetes incertae sedis*, with high similarity to *Microdochium*. In Ecuador, *Paraphaeosphaeria* is mostly present in the highlands and almost absent in the Amazon region. In Guatemala, *P. maydis* was the most abundant taxon in two locations but was almost absent in a third. The frequency of *Coniothyrium* in all locations was less than one percent. Our results show that the composition of fungal communities associated with tar spot is determined by geographic location and that *Microdochium* likely plays a significant role in disease development in Ecuador and Guatemala.

Tar spot of corn, *Microdochium maydis*, *Coniothyrium phyllachorae*, microbiome, biological control.

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38. Large-scale root microbiome profiling identifies groups of variety-specific microbes with important functional potential for barley

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Understanding how root microbiomes interact with crop varieties can identify novel breeding strategies that target beneficial interactions between the host plant and microbes. This requires deep insight into host-microbe interactions, especially across a large diversity of varieties. In the Horizon-Europe-funded BarleyMicroBreed project, we analyzed 16S and ITS amplicon sequencing data from 3,600 root microbiomes across 600 field-grown barley varieties representing panels of spring- and winter barley, and in addition a diversity panel encompassing barley introgressions that were backcrossed between ancient and modern barley (i.e., *Hordeum spontaneum* and *H. bulbosum*). Our analysis on spring and winter panels showed that barley variety explained the largest variation within bacterial and fungal communities (ca. 20% - 30%), compared to other factors like geospatial heterogeneity (ca. 2% -

5%). Surprisingly, over 70% of the bacterial ASVs and 57% of fungal ASVs were genotype-specific (specific to fewer than 20% of varieties), contrasting with the core microbiome (i.e., shared by over 80% of varieties) accounting for only 8% and 15% of the ASVs for bacteria and fungi, respectively. The taxonomic analyses showed that even at the class level, around 40% of bacterial classes and 25% of fungal classes were genotype-specific. These genotype-specific ASVs collectively represented 23% of the total relative abundance of bacteria and 10% of fungi. Functional prediction by PICRUST2 on bacterial communities indicated that the predicted KEGG orthology of genotype-specific ASVs exhibited similar variation between genotypes in microbiome functioning as observed in core microbiomes. Moreover, the functional potential inferred from genotype-specific ASVs significantly differed from that of the core microbiomes. Given the high number and abundance, and various functional potentials of these genotype-specific microbes, they emerge as an important microbial resource for promoting barley phenotypes. Such insights may inform novel breeding strategies tailored to harness the benefits of indigenous soil microbial diversity.

Barley genotype, root microbiome, genotype-specific microbes

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39. The interplay between the inoculation of plant growth-promoting rhizobacteria and the rhizosphere microbiome and their impact on plant phenotype

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Microbial inoculation stands as a pivotal strategy, fostering symbiotic relationships between beneficial microorganisms and plants, thereby enhancing nutrient uptake, bolstering resilience against environmental stressors, and ultimately promoting healthier and more productive plant growth. However, while the advantageous roles of inoculants are widely acknowledged, the precise and nuanced impacts of inoculation on the intricate interactions of the rhizosphere microbiome remain significantly underexplored. This study explores the impact of bacterial inoculation on soil properties, plant growth, and the rhizosphere microbiome. By employing various bacterial strains and a synthetic community (SynCom) as inoculants in common bean plants, the bacterial and fungal communities in the rhizosphere were assessed through 16S rRNA and ITS gene sequencing. Concurrently, soil chemical parameters, plant traits, and gene expression were evaluated. The findings revealed that bacterial inoculation generally decreased pH and V%, while increasing H+Al and m% in the rhizosphere. It also decreased gene expression in plants related to detoxification, photosynthesis, and defense mechanisms, while enhancing bacterial diversity in the rhizosphere, potentially benefiting plant health. Specific bacterial strains showed varied impacts on rhizosphere microbiome assembly, predominantly affecting rhizospheric bacteria more than fungi, indirectly influencing soil conditions and plants. Notably, *Paenibacillus polymyxa* inoculation improved plant nitrogen (by 5.2%) and iron levels (by 28.1%), whereas *Bacillus cereus* boosted mycorrhization rates (by 70%). Additionally, inoculation led to increased complexity in network interactions within the rhizosphere (~15%), potentially impacting plant health. Overall, the findings highlight the significant impact of introducing bacteria to the rhizosphere, enhancing nutrient availability, microbial diversity, and fostering beneficial plant-microbe interactions.

Common bean; Microbial ecology; 16S rRNA; ITS

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40. A plant vaccine protecting tomato from Pepino Mosaic Virus in over 20 countries

Pedro Beschoren da Costa, Adrianus Vermunt, and Thorben Looije

Viruses are a critical but neglected part of the plant holobiont. Viewing them exclusively as agents of plant disease is an outdated misconception from the previous century. It has been shown that they can heavily impact other members of the plant holobiont directly or indirectly. Some of these interactions can be leveraged for sustainable agriculture, such as cross-protection effects where infection with one viral strain prevents subsequent establishment of another. This processes has been demonstrated in Pepino Mosaic Virus (PepMV) infecting four tomato cultivars in six greenhouse trials, with four replicates per treatment and cultivar combination. In the cross-protection treatment, the mild PepMV strains VX1 and VC1 were inoculated in 3-week old tomato plants, while the aggressive PepMV strains virEU and virCH were inoculated when the same plants were six weeks old. Control treatment plants were inoculated with only the aggressive strains, only the mild strains, or a mock solution with no active strains. The cross-protection treatment significantly reduced various PepMV symptoms when compared to the controls with aggressive strains only. Nettle head reduced from 58 to 5%, stunting reduced from 55 to 2%, blotchy fruits reduced from 22 to 2%, leaf necrosis reduced from 58% to 3%, stem necrosis reduced from 25 to 1%, while harvested fruit fresh weight increased from zero to 9.5kg. Plants with the mild strains presented very mild and temporary symptoms (less than 5%). This mixed strain treatment was successfully formulated into a product, registered, and commercialized by Valto Biocontrol as a plant protection product named “V10”. Today this product is exported to more than 20 countries inside and outside the EU, targeting greenhouse tomato cultivation. The company now aims to expand its product range and is interested in stablishing collaborations to explore new virus-crop pathosystems, emerging plant viral pandemics, and virus-focused biocontrol products.

Solanaceae, Phytopathology, biocontrol, cross-protection, virome

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41. Identification of bacterial indicators for disease suppressive composts in three different pathogen – plant systems

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Composts can suppress soil-borne diseases, although their effectiveness varies across combinations of pathogen-plant systems and composts. The underlying mechanisms, likely driven by compost microorganisms, are not fully understood. Identification of microorganisms responsible for disease-suppression with compost is crucial for its targeted use in plant protection. We tested 37 composts from commercial compost producers for their abiotic properties, microbial activity and disease suppressiveness in the three pathogen-plant systems: I) *Globisporangium ultimum* (GU)-cress, II) GU-cucumber and III) *Rhizoctonia solani* (RS)-cucumber, and analyzed their bacterial communities using metabarcoding of the 16S rRNA gene. Of the 37 composts tested, 76%, 46% and 24% significantly increased disease suppression in the three respective systems. No significant correlation with disease

suppression was found for the abiotic properties and microbial activity of the composts. Bacterial ASV-richness of composts correlated significantly with disease suppression in GU-cress ($\rho = 0.50$, $p = 0.002$). In the two cucumber assays, a similar but less significant trend was noted. The bacterial community structures of the composts did not correlate with disease suppressiveness. Indicator analysis on genus level (ASVs classified using Genome Taxonomy Database) revealed a total of 108 bacterial genera, i.e., 51 for GU-cress, 40 for GU-cucumber and 44 for RS-cucumber, that were significantly more abundant in the most suppressive composts. Fourteen indicative genera were shared between GU-cress and GU-cucumber, while between these two and RS-cucumber only 7 and 4 were shared. The genus *Algoriphagus* (in both GU-systems) and the genus *Sphingopyxis* (only in GU-cucumber) have been identified as indicative for suppression of GU in cress in a previous study. In conclusion, abiotic properties, microbial activity and the bacterial community structure of the composts do not explain differences in their disease suppressiveness. However, certain bacterial taxa were consistently more abundant in the most suppressive composts of specific pathogen-plant systems.

Compost, disease suppression, 16S metabarcoding, indicator taxa, *Globisporanigum ultimum*, *Rhizoctonia solani*

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42. Localization of antimicrobial compounds in plant organs of *Pimpinella saxifraga*

M. HANSER, C.-H. HSIEH, S. HAN, F. S. HANSCHEN, K. WITZEL

Wild plant species play an important role in the ecosystem but are also utilized due to their bioactive compounds. We have investigated *Pimpinella saxifraga*, a perennial herbaceous plant from the Apiaceae family. Roots and leaves are used in traditional medicine. So far, mainly the essential oil of the seeds has been investigated for antimicrobial activity. In this study, extracts of various organs were examined for their antimicrobial effect.

Ethanollic plant extracts of *P. saxifraga* have been screened in vitro in bioassays for their antimicrobial activity against the soilborne pathogenic fungi *Rhizoctonia solani* and the leaf pathogenic bacteria *Xanthomonas campestris*. The highest antimicrobial properties were found for the seeds and roots. In order to identify the respective bioactive compounds, the extracts were fractionated via solid phase extraction and preparative HPLC. Structural analysis by nuclear magnetic resonance was used to identify the antimicrobial compound.

To further explore the localization and distribution of the antimicrobial compounds in plant tissues, we examined the seeds of *P. saxifraga* during the germination process. It could be shown that the antimicrobial compounds remained largely in the seed coat and were less detectable in the seedling root and the seedling shoot.

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antimicrobial compound, *Pimpinella saxifraga*

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43. Characterization of American strains of *Colletotrichum tofieldiae*.

Díaz-González, S., Marín, P., Pérez, R., Olivares, P., Donohue, K., Brunner, F., Borja, M. and Sacristán, S.

Characterization of American strains of *Colletotrichum tofieldiae*.

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Plant Response

Spain

Session 2: Plant-microbiome communication and assembly

44. Plant-driven assembly of downy mildew-suppressive microbiomes

J. Spooren, P. Goossens, D. Lapin, C.M.J. Pieterse, G. Van den Ackerveken, R.L. Berendsen

Plant microbiomes can be dynamically manipulated by the plant in response to pathogen attack to enhance plant resistance. As downy mildew pathogens are obligate biotrophs, they populate living plant hosts where they co-occur with other leaf microbiota. In the laboratory, the downy mildew of *Arabidopsis thaliana*, *Hyaloperonospora arabidopsidis* (Hpa), is cultured by successive weekly passaging of spores from diseased to healthy host plants. We hypothesized that this leads to the selection of a disease-associated microbiome that mediates the interaction between the plant and the pathogen. Here we show that Hpa-infected plants assemble specific beneficial bacteria that accumulate in Hpa cultures, reduce disease and can be inherited as a soilborne legacy. We found that distinct Hpa cultures, derived from multiple isolates maintained separately in British, German and Dutch laboratories, were dominated by isogenic bacteria. These Hpa-associated microbiota (HAM) thrive in the environment of the infected plant but negatively affect pathogen proliferation. Moreover, we demonstrated that plants, upon infection with gnotobiotic Hpa spores in which the HAM are absent, specifically recruit HAM from natural soil. Finally, the HAM can persist in the soil as a soilborne legacy, dominating the phyllosphere of subsequent plant populations growing on soil conditioned by (gno)Hpa inoculated plants. Our results indicate that upon initial infection and consecutive passaging of downy-mildew, plants assemble a pathogen associated but disease-suppressive microbiome, which was dubbed a 'resistobiome'.

resistobiome, soilborne legacy, downy-mildew, microbiome assembly, resistance

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45. Do plant polysaccharides influence microbiome assemblage?

Hannah Martin, Ian Lidbury, Sam Amsbury, Tim Daniell

"Through the release of exudates, plants manipulate the soil surrounding their roots, creating a region called the rhizosphere. Microbiota inhabiting the rhizosphere are typically well adapted to utilise these root exudates, which are composed of low molecular weight and high molecular weight (HMW) molecules. Whilst there have been significant advances toward understanding the composition of the plant rhizosphere microbiome, the importance of HMW polymers, e.g., polysaccharides, in driving assemblage is very limited. Bacteroidota represent an abundant

bacterial phylum found in the soil and are typically enriched in the plant microbiome. In the human gut, Bacteroidota are key polysaccharide degraders, including various dietary plant polysaccharides. However, the role of soil and plant-dwelling Bacteroidota on plant polysaccharides has received less attention. Bacteroidota possess specialised gene clusters, termed polysaccharide utilisation loci (PUL). These PULs encode proteins with functions relating to the utilisation of complex carbohydrates. However, the exact mechanisms enabling Bacteroidota to degrade complex carbohydrates in the soil and succeed in this niche are unknown.

My work has identified specific PULs associated with plant polysaccharide utilisation and investigated the role this unique metabolism has on plant microbiome assemblage. Using *Flavobacterium* spp. as the model and combining bacterial genetics, proteomics and light microscopy, I have shown plant polysaccharide utilisation provides *Flavobacterium* with a competitive advantage when inhabiting the plant microbiome. Given *Flavobacterium*, as well as other Bacteroidota have an important role in plant disease suppression, I plan to further investigate any direct or indirect interactions occurring between *Flavobacterium* and bacterial root pathogens, such as *Xanthomonas* and *Ralsontia*."

Polysaccharides, *Flavobacterium*, Bacteroidota, Exudates, Assemblage, Proteomics

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46. A defense protein mediates microbiome feedbacks in Arabidopsis

Henry Janse van Rensburg, Niklas Schandry, Claude Becker, Klaus Schlaeppi

Plants secrete a diverse array of compounds into the soil to condition the surrounding microbiome. Root exudation often results in plant soil feedbacks, that define the performance of the second plant generation. Benzoxazinoids (BXs) are plant toxins abundant in the root exudates of important crops like maize and wheat. The rhizosphere microbiota is selectively structured by the exudation of BXs, mediating growth and defense feedbacks on subsequent maize and wheat generations. While the ecology and agronomic impact of plant-soil feedbacks in crop rotations are well described, little is known about the underlying mechanisms of plant responses to soil microbiomes. Natural *Arabidopsis thaliana* accessions show a tremendous diversity in their growth feedback when grown on a BX-conditioned microbiome. Using a Genome Wide Association study performed on 410 of these *Arabidopsis* accessions, we identified a gene that codes for a toll-interleukin receptor nucleotide-binding site leucine-rich repeat protein (TNL), called mediator of microbiome feedbacks 1 (MMF1), to be associated with the BX microbiome-driven growth feedback. We showed that plants that lack a functional copy of MMF1 no longer show a positive growth feedback when grown on a BX-conditioned soil microbiome. Microbiome profiling revealed that mutant plants assemble a small but significantly altered bacterial root microbiome compared to wild-type plants. Transcriptomics performed on the roots showed that *mmf1* mutant plants are transcriptionally unresponsive to a BX-conditioned soil microbiome. Our results show that MMF1 acts as a key regulator of BX-mediated microbiome feedbacks in *Arabidopsis*.

Plant-soil feedbacks, microbiome, GWAS, *Arabidopsis*, TIR-NBS-LRR

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47. Molecular investigation of an atypical mechanism for organophosphorus uptake in plant-associated Flavobacterium

Alex Connolly, Laila Moushtaq, Stephen Rolfe, Andrew Hitchcock, Ian Lidbury

Phosphorus is a major limiting nutrient for microbial growth in the rhizosphere, being mostly in inaccessible forms, such as in insoluble minerals or organophosphorus; both of which require conversion into inorganic phosphate for use in central metabolism. Under phosphorus stress, soil bacteria typically express high-affinity ATP-binding cassette transporters to scavenge inorganic phosphate from the limited amount present. Flavobacterium spp. of the phylum Bacteroidota, which are enriched in the rhizosphere compared to bulk soil, lack these, instead expressing SusCD-like TonB-dependent transporters under phosphorus stress. We hypothesise that these SusCD-like transporters import organophosphorus into the Flavobacterium periplasm, where it is subsequently broken down into inorganic phosphate by phosphatases. Previously, only SusCD-like transporters involved in polysaccharide uptake have been characterised experimentally. Here, we show that one of these phosphorus stress-inducible transporters likely binds and imports phosphoinositides: organophosphorus compounds derived from eukaryotic membranes.

Flavobacterium, Bacteroidota, rhizosphere, organic phosphorus, phosphatidylinositol, TonB-dependent transporters

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48. Analysis of bacterial degradation specificity toward soybean-derived isoflavones and effects of those metabolites on soil bacterial community structure

Tomoaki Sato, Kyoko Takamatsu, Hinako Matsuda, Noritaka Aoki, Akinori Ando, Shigenobu Kishino, Jun Ogawa, Sachiko Masuda, Arisa Shibata, Ken Shirasu, Tomohisa Shimasaki, Kazufumi Yazaki, Akifumi Sugiyama

"Soybean roots secrete a variety of isoflavones into the rhizosphere. Daidzein, a major soybean isoflavone, serves as a signaling molecule for rhizobial symbiosis. In the daidzein-treated soil, the relative abundance of Comamonadaceae increased and shifted soil microbial community like that of soybean rhizosphere soil, suggesting that isoflavones are involved in the regulation of the soybean rhizosphere microbiota. Understanding isoflavone catabolism in soybean rhizosphere will provide a deeper insight into the molecular mechanisms of soybean-rhizosphere microbial interactions. We previously isolated daidzein-degrading bacteria from soybean roots, Variovorax sp. strain V35 and Acidovorax sp. strain A79, both of which belong to the family Comamonadaceae. Further, we discovered an isoflavone catabolism (ifc) gene cluster in strain V35 by which isoflavones are oxidatively catabolized. In this study, we conducted a degradation assay of various rhizosphere bacteria using isoflavones of different structures to elucidate the molecular mechanisms underlying the microbial-mediated metabolic network of isoflavones in soybean rhizosphere.

To verify the specificity of the isoflavone degradation capacity of three types of soybean rhizosphere bacteria: strain V35, strain A79, and Bradyrhizobium diazoefficiens strain USDA110, we analyzed degrading activities using three isoflavones: daidzein, genistein, and

glycitein. The results showed that daidzein-degrading bacteria have different degradation abilities toward glycitein and strain USDA110 didn't exhibit the isoflavone degradation activity.

We also performed 16S rRNA amplicon sequencing analysis on the extracted DNA from soil-treated with three types of isoflavones and found the distinctive communities of glycitein-treated soil, suggesting that glycitein may have different impacts on the assembly of soybean microbial community. We are currently evaluating the specificity of isoflavone degradation in isolates belonging to ten families from soybean roots by isoflavone-enrichment culture and conducting comparative genomic analysis."

Isoflavone, Rhizosphere, Soybean, Isolation, Bacteria, Metabolism

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49. Molecular and chemical cues in the endophytic microbiome

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We recently discovered that plants under attack by fungal and oomycete pathogens can actively recruit endophytic microbes inside their root tissues (endosphere) and on their leaves (phyllosphere) for protection. We showed that *Cupriavidus* species were significantly enriched in the root endosphere of sugar beet upon belowground *Rhizoctonia solani* infection, while *Xanthomonas* species were significantly enriched in the phyllosphere of *Arabidopsis thaliana* upon aboveground downy mildew (*Hpa*) infection. The molecular mechanisms for such recruitment are yet unknown. We are using transposon insertion mutagenesis strategies (RB-TnSeq, INSeq) in plant-derived *Cupriavidus* and *Xanthomonas* isolates to identify genetic factors involved in this specific recruitment and the more general endophytic lifestyle. Our results and ongoing experiments will shed light on microbiome assembly in the endosphere and phyllosphere of plants under siege.

Endophytes, TnSeq, disease suppression

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50. Improvement of *Lotus japonicus* root synthetic community using hybrid de-novo genome assembly and computational inspection of genetic features.

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***Corresponding author: Adrián Gómez-Repollés**

The study of plant-associated bacterial strains and their role in plant-bacteria or bacteria-bacteria interaction benefits from high quality genomic and genetic data. For example, the identification of gene clusters (e.g. secondary metabolites or secretion systems) profits from the correct location of genes in continuous genomes when performing genome mining. To better understand the previously isolated *Lotus japonicus* root-associated commensal bacterial synthetic community (from now on LjSC), we performed long read sequencing (Nanopore: MinION) on a target subset of the previously isolated LjSC bacteria strains. Bacterial genomes were computationally reconstructed using a pipeline performing an hybrid

assembly approach combining the newly produced long reads and the previous short reads (Illumina: HiSeq-2500). The resulting assemblies were followed, when needed, by manual curation supported by computational analysis. The produced genomes were annotated and validated before continuing with downstream analysis, such as, taxonomy categorization, plasmid identification and genome mining of relevant targets. We obtained 144 high quality genomes including their annotation and taxonomy. The majority of the genomes belonged to the phylum Proteobacteria but the overall taxonomic diversity was rich. Family-wise, there were 3-4 major families gathering a large number of the total genomes. Further, around 1/3 of the genomes contained plasmids with dispersed sizes but showing overlap across genomes of the same family. In addition, there was a rich diversity of secretion and toxin-antitoxin systems identified across the genomes. In brief, the improved bacterial references from LjSC constitute a unique genomic/genetic dataset that would be beneficial for analysis such as, metagenomics or transcriptomics, but also bacteria root-associated gene identification."

Lotus japonicus, synthetic community, hybrid assembly, gene clusters

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51. Phyllosphere bacterial dynamics and community assembly in a temperate forest

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Phyllosphere microbiomes are subject to receive imports from various sources and undergo succession through phenological changes of the plant host. Phenology-associated microbiome dynamics are less well understood for trees and it is unclear whether host identity or stochastic processes control bacterial community assembly. We leveraged the Leipzig Canopy Crane Facility located in a temperate floodplain forest in Germany to (1) assess successional changes (comparing May, July and October 2021) and throughfall-mediated vertical transfer (top, mid and bottom canopy positions) of phyllosphere bacteria for *Quercus robur* L. (English oak), *Fraxinus excelsior* L. (European ash), and *Tilia cordata* Mill. (Small-leaf European linden), and (2) identify the dominant community assembly processes. More Amplicon Sequence Variants (ASVs) were shared between throughfall and phyllosphere in May and October (10 – 23%) than in July (4 – 9%), suggesting stronger mobilization of phyllosphere bacteria by precipitation at early and late phenological stages. Bacterial colonization at the leaf sprouting stage was dominated by airborne dispersal (e. g., *Erwiniaceae*) and pollen-mediated transport (e. g., *Pseudomonadaceae*). Tree species-specific influence increased during July and October especially for linden with a dominance of typical phyllosphere taxa such as *Beijerinckiaceae* and a higher community overlap between July and October (22 – 45%) compared to May and July (16 – 30%). Drift (25 – 54%) and dispersal limitation (10 – 52%) were the dominant assembly processes, most likely associated with stochastic weather events, while homogeneous selection as deterministic assembly processes pointed to host-mediated controls and was specifically linked to *Beijerinckiaceae*. Our results suggest that rainwater-driven microbial transport plays a crucial role for the import of bacterial taxa to phyllosphere and for their establishment and redistribution. Stochastic processes such as drift and dispersal limitation determine phyllosphere bacterial community during the sprouting stages, while *Sphingomonadaceae*-driven drift becomes the dominant assembly process during later successional stages.

Phyllosphere; throughfall; temperate forest; drift and dispersal limitation

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52. Medicago truncatula genotype drives the plant nutritional strategy and its associated rhizosphere bacterial communities

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"With the growing concern of developing a more sustainable agriculture, decreasing the use of inputs, and promoting biological diversity, harnessing plant microbiome through plant genetics is gaining of interest to improve plant growth, nutrition, and health. While genome-wide association studies have been conducted to identify plant genes driving the plant microbiome, more multidisciplinary studies are required to assess the relationships among plant the genetic effects, the plant microbiome and plant fitness.

Using a metabarcoding approach, we characterized the rhizosphere bacterial communities of a core collection of 155 Medicago truncatula genotypes together with the plant phenotype, using an ecophysiological framework, and investigate the plant genetic effects through genome-wide association studies.

The different genotypes within the M. truncatula core collection showed contrasted growth and carbon and nitrogen nutritional strategies but few loci were associated to these ecophysiological traits. To go further, we described its associated rhizosphere bacterial communities, dominated by Proteobacteria, Actinobacteria and Bacteroidetes, and defined a core rhizosphere bacterial community. Next, occurrence of bacterial candidates predicting plant ecophysiological traits of interest were identified using random forest analyzes. Some of them were heritable and plant loci were identified, pinpointing genes related to response to hormone stimulus, systemic acquired resistance, response to stress, nutrient starvation or transport, and root development.

Together, these results suggest that plant genetic can affect the plant growth and nutritional strategies harnessing keystones bacteria in a well-connected network community."

Bacterial communities, GWAS, Medicago truncatula, plant nutritional strategy, rhizosphere

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53. Plant transcription factors in the assembly of host species-specific microbial communities

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The plant root-associated microbiota benefits plants via growth promotion, nutrient uptake facilitation, and stress alleviation. These benefits depend on the composition, diversity, and abundance of microbiota. The assembly of the root-associated microbiota has been shown to be affected by the genetic makeup of the host plants, chemical and physical properties of the bulk soil, diversity and composition of the soil microorganisms. However, we still lack detailed knowledge about which host genes are involved in such progress. The aim of our project is to identify and functionally characterize plant host factors that are involved in the assembly of

host-specific root-associated bacterial communities. Recently, using gnotobiotic growth systems with synthetic bacterial communities (SynComs), it was demonstrated that diverse root-associated bacteria isolated from *Arabidopsis thaliana* (At) and *Lotus japonicus* (Lj) form distinct communities on roots, and preferentially colonize their cognate host. Host responses were detected via RNA-seq after separate inoculation with either At or Lj SynComs.

Homologous transcription factors (TFs) that were co-upregulated in different host species by their respective cognate SynCom were selected as candidate genes. Mutant lines of At and Lj are utilized to test plant performance and assess microbiota composition upon co-inoculation with mixed and separate At and Lj SynComs. Differences in microbiota composition between mutant and wt plants and between host species will reveal a role of the TF in host-specific microbiota assembly. Additionally, chemical analysis of root exudate composition and biochemistry assays for functional characterization of the TFs will be performed. This will identify downstream targets regulated by these TFs. Our findings contribute to the understanding of host genes involved in root microbiota establishment.

root-associated microbiota, *Arabidopsis*, *Lotus*, transcription factors, metabolites

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54. Genome-resolved Metagenomics of Litter's Undergoing Forest-to-Pasture Conversion and Forest Recovery

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Previous findings have demonstrated that soil microbial communities exhibit responses to forest-to-pasture conversion and forest recovery in the Amazon rainforest. However, the identities and ecological roles of the majority of these organisms remain unknown. Here, we used genome-resolved metagenomics to assemble and retrieve metagenome-assembled genomes (MAGs) from litter samples collected from primary forest, pasture, and regenerating forest in the Eastern Amazon. Samples were collected from primary forest, pasture, and secondary forest in Tomé-Açu, State of Pará, Brazil. These samples underwent chemical analysis of litter plant tissue for determine the levels of elements, DNA extraction, and shotgun metagenomic sequencing using an Illumina platform. On the KBase platform, we conducted quality control of sequences (Trimmomatic), metagenome assembly (MEGAHIT) and binning (MetaBAT2, MaxBin2, CONCOCT and DAS Tool). This was followed by quality assessment (CheckM), taxonomic classification (GTDB-Tk) and functional annotation (DRAM). We obtained 29 medium- and high-quality MAGs across five microbial phyla (medium: $\geq 50\%$ completeness and $< 10\%$ contamination, high: $> 90\%$ completeness and $< 5\%$ contamination). Of these, 13 were from forest, 12 from pasture, and 4 from secondary forest. Specifically, 7 high-quality MAGs from the primary forest included Bacteroidota (4), Actinomycetota (1), Pseudomonadota (2). From the pasture, 9 high-quality MAGs included Bacteroidota (2), Actinomycetota (1), Pseudomonadota (4), Acidobacteriota (1), and Bacillota (1). 1 high-quality MAG from the secondary forest was identified as Bacteroidota. Several functional characteristics were detected in these MAGs, including genes involved in carbon and nitrogen cycles, and those associated with carbohydrate degradation, crucial for organic matter cycling and plant growth. Genome-resolved metagenomics can expand our knowledge of the microbial communities of Amazonian forests and pastures, thereby revealing the functional potential of new microorganisms and the plant microbiome, elucidating their role in biogeochemical cycles, and

increasing information for cultivation-dependent techniques, including in areas undergoing plant regeneration.

Amazon rainforest. Forest-to-pasture conversion and Forest recovery. Litter microbial communities. Plant decomposition. Metagenomic sequencing. Metagenome-assembled genomes.

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55. Plant microbiota feedbacks through tunable expression of core immunity genes

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The perception of, and reaction to, biotic and abiotic stresses is critical for plant health. We recently identified a core set of genes consistently induced by members of the leaf microbiota, termed general non-self response (GNSR) genes. Here, we show that GNSR components conversely impact leaf microbiota composition. Specific strains that benefitted from this altered assembly triggered strong plant responses, suggesting that the GNSR is a dynamic system that modulates colonization levels of certain strains. Examination of the GNSR to live and inactivated bacteria revealed that bacterial abundance, cellular composition, and exposure time collectively determine the extent of the host response. We link the GNSR to pattern-triggered immunity (PTI), as diverse microbe- or danger-associated molecular patterns cause highly dynamic GNSR gene expression. Our findings suggest that the GNSR is the result of a fine-tuned perception and signaling system that feeds back to the leaf microbiota and contributes to the intricate balance of plant-microbiome interactions.

phyllosphere microbiota, plant-microbe interactions, plant perception, immune signaling, microbiota homeostasis, pattern-triggered immunity

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56. Identification of signaling compounds influencing root colonization by ascomycetes

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Plants spend up to a third of their energy on the production of metabolites that are then exuded from the roots. These so-called root exudates act to shape the rhizosphere to the needs of the plant. For example, by modifying soil properties to be more favorable, but also by attracting and promoting the growth of certain microbes, leading to the establishment of a unique root microbiome. Root colonizing fungi are an important part of the root microbiome. Trichoderma species can benefit the plant by facilitating nutrient uptake and attacking pathogens, whereas certain varieties of Fusarium species are pathogenic and can lead to devastating agricultural

losses. For root colonization to occur, the fungus first has to locate a root. This process is currently poorly understood, but can be divided into three steps. Namely, (1) a mixture of chemicals is exuded from the plant root, the composition of which changes depending on growth and stress conditions, (2) some of these chemicals are perceived by the fungus, and (3) the fungus responds in a manner that makes it more likely to encounter a root. Here, we investigate what kinds of chemicals are exuded from lettuce during nitrogen or phosphate deficiency, and which receptors the root colonizing fungi *Fusarium oxysporum* and *Trichoderma harzianum* use to perceive these signals, and how they respond to these signals. As of yet, root exudates have been collected from nitrogen or phosphate deficient aeroponically grown lettuce, and are being investigated for the presence of oxylipins—a type of chemical that elicits directed growth in ascomycetes. Furthermore, a bioinformatics pipeline using hidden Markov models was constructed for high-throughput cataloging of G-protein coupled receptors (GPCRs) in fungal genomes, which has already revealed thousands of candidates across *Trichoderma* and *Fusarium* species. GPCRs are vital for responses to environment and host. Finally, biological responses of fungi to lettuce root exudates are being tested.

Plant-fungi signalling, Root exudates, Root colonizing fungi, Root sensing, *Fusarium*, *Trichoderma*, Oxylipins, G-protein coupled receptors, nutrient stress.

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57. Volatile-mediated recruitment of beneficial rhizobacteria by tomato plants under herbivory stress

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Plant roots release multiple volatile organic compounds (rVOCs) important for belowground chemical communication. External stress stimuli such as leaf herbivory can locally or systemically change root volatile emission, both quantitatively and qualitatively. Such stress-induced volatiles can have direct defensive effects against invading pathogens or insect pests, while others act as chemical cues attracting or activating specific beneficial (micro)organisms for additional protection. To date, however, it is not known if and how volatile emissions from roots of plants under stress lead to the recruitment of specific members of the soil microbiome. We previously showed that tomato plants suffering from leaf herbivory by *Spodoptera exigua* emit different profile of root volatiles than non-stressed plants. Next, we applied a belowground olfactometer system to investigate if these stressed tomato plants can recruit, via these differential root volatiles, a specific subset of soil bacteria. We observed significant changes in the root volatilome of *Solanum lycopersicum* upon herbivory stress and a concomitant change in the taxonomic composition of the rhizobacterial communities. After targeted isolation of several stress-recruited rhizobacteria, we confirmed that several of these species/strains provide protection against the herbivory stress. Furthermore, we found that methyl salicylate, one of the identified stress-related rVOCs, can induce surface motility and biofilm formation in some of the recruited rhizobacterial species, indicating that this specific volatile contributes to rhizosphere microbiome assembly of tomato plants under siege.

root volatiles, *Solanum lycopersicum*, insect herbivory, olfactometer, belowground plant-microbe interaction

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58. 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. LC-MS/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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59. 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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60. The dominant role of *Lactuca sativa* morphological traits over genotype in shaping leaf-associated bacterial community

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Lettuce is one of the main vegetables consumed raw worldwide. Studying the driving forces that shape the leaf-associated bacterial community can provide a means to examine the establishment of a beneficial microbial population essential for both plant health and human nutrition. This project aims to uncover the influence of plant determinants, such as genetic distance, leaf mineral content and leaf morphology, in impacting diversity, richness and association of lettuce leaf bacteria.

To address these knowledge gaps, we conducted a large-scale experiment utilizing 131 fully-sequenced genotypes of *Lactuca sativa*, cultivated over three months, and investigated their leaf-associated bacterial communities, using DNA amplicon sequencing of the bacterial 16SrRNA.

Results demonstrate that genetic distance and plant morphology (defined by breeders as “variety”) are responsible for 3.3% and 4.4% of the overall leaf-associated bacterial community diversity, respectively. In addition, disparities in manganese (Mn) concentration among groups of closely related genotypes, as well as variations in calcium (Ca), zinc (Zn), and phosphorus (P) among different varieties, contribute to explaining this variation. Variety's importance prompted us to incorporate extensive phenotypic traits, identifying primary influencers and mechanisms. We identified 10 leaf traits that affect the structure of the bacterial community, with heart formation, head height and shape impacting bacterial richness and evenness. Altogether, these different factors explain 13.6% of the leaf-associated bacterial diversity. Finally, the origin of the leaf bacterial community was analyzed by assessing the proportion of bacteria originating from various environmental sources. Interestingly, seed-associated bacteria were found to mostly contribute to the leaf community composition.

By investigating how variation in phenotypic appearance leads to the establishment of diverse prokaryotic communities, we disentangled previously unknown mechanisms through which plants influence the recruitment of leaf bacteria, offering new observation lens for breeders and novel insights into similar associations in other agriculturally relevant crops.

phyllosphere, genetic variation, plant morphology, plant nutrition

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61. Cope and communicate – Spatial interplay between plant specialized metabolites, physical barriers and the root microbiome

Pascal Krohn, Tonni Grube Andersen

In response to challenging environments plants have evolved a diverse arsenal of physical and chemical defense mechanisms to ensure growth and propagation. Brassicales species, including the model plant *Arabidopsis thaliana*, use a set of specialized metabolites termed glucosinolates (GSL). These compounds serve as a major chemical defense system, which can be spatially employed across different tissues and plays clear roles in plant defense against herbivores and pathogenic microbes. However, most of the underlying studies have been performed in aboveground tissues and we lack understanding of spatial GSL deployment in roots. Physical protection belowground on the other side is better understood. Along the root, physical barriers range from the highly suberized and lignified periderm at the top to a very plastic, neither suberized nor lignified state, at the root tip. However, both physical and chemical barriers need to be spatially aligned to provide an effective defense. Intriguingly, recent data also shows that the root microbiome is organized along the longitudinal root axis, suggesting a yet to be characterized interplay between microbes, chemical and physical root barriers.

Inspired by this, we set out to investigate connections between physical and chemical barriers and how they are linked to root microbiota assembly. We deploy a multi-omics approach on distinct root sections combining transcriptomics, metabolomics on root tissue and exudates as well as microbiome profiling. A deeper insight into the crosstalk between chemical and physical defense strategies and how these reshape the rhizosphere would enable us to better understand mechanisms that control beneficial microbiome recruitment as well as the detrimental effects of microbial dysbiosis in the soil.

Spatial, Root, Barriers, Glucosinolates

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62. Maize root bacteria degrade host-specialized metabolites through the lactonase BxD A

Christine Pestalozzi, Lisa Thoenen, Anlun Wei, Caitlin Giroud, Klaus Schlaeppi

Root exudates contain specialised metabolites that shapes the plant's root microbiome. How host-specific microbes cope with these bioactive compounds, and how this ability affects root microbiomes, remains largely unknown. We investigated how maize root bacteria metabolise benzoxazinoids, the main specialised metabolites of maize. Diverse and abundant bacteria metabolised the major compound in the maize rhizosphere 6-methoxy-benzoxazolin-2-one

(MBOA) and formed 2-amino-7-methoxy-phenoxazin-3-one (AMPO). AMPO forming bacteria are enriched in the rhizosphere of benzoxazinoid-producing maize and can use MBOA as carbon source. We identified a novel gene cluster associated with AMPO formation in microbacteria. The first gene in this cluster, *bxDA* encodes a lactonase that converts MBOA to AMPO in vitro. A deletion mutant of the homologous *bxDA* genes in the genus *Sphingobium*, does not form AMPO nor is it able to use MBOA as a carbon source. *BxDa* was identified in different genera of maize root bacteria. Here we show that plant-specialised metabolites select for metabolisation-competent root bacteria. *BxDa* represents a novel benzoxazinoid metabolisation gene whose carriers successfully colonize the maize rhizosphere and thereby shape the plant's chemical environmental footprint.

benzoxazinoids, host-specialized metabolites

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63. Diversification of strigolactones in Cucurbitaceae and its effects on arbuscular mycorrhizal fungi symbiosis

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Symbiosis with arbuscular mycorrhizal fungi (AMF) is an ancient and conserved trait in land plants. It involves trading carbohydrates and lipids produced by the plant for nutrients, mainly phosphorus and nitrogen, acquired by the fungi. Under phosphate deficiency conditions, plants initiate the process of symbiosis and recruit AMF by exuding strigolactones, a class of signaling molecules derived from the beta carotene biosynthesis pathway. So far, more than 25 unique molecular structures have already been described in the literature in many plant species. However, the evolution of strigolactones and the effects of domestication on their structural diversification and exudation remain unclear. Using a characteristic fragmentation pattern in mass spectrometry, we developed a high-throughput strigolactones identification pipeline. This allowed us to identify several previously undescribed strigolactones in 16 Cucurbitaceae species. Mapping these strigolactones to the phylogenetic tree of Cucurbitaceae, we identified diversification events in the evolutionary history of this plant family. We also highlighted the effects of evolution and domestication on strigolactones exudation patterns across the Cucurbitaceae phylogeny. Finally, we measured the relationship between strigolactones and AMF community composition in the roots under varying stress conditions.

Arbuscular Mycorrhizal Fungi, Strigolactones, Evolution, Domestication

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64. Root metabolites shape the rhizosphere microbiome of Solanaceae plants under drought

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Drought is a major factor limiting crop production and has been increasing in frequency, duration and intensity through climate change. One promising strategy to face this challenge is to exploit the benefits of the rhizosphere microbiome. This microbiome can contribute to plants' resilience to various biotic and abiotic stresses, including drought, through a multitude

of mechanisms. In this study, we used amplicon sequencing to explore the bacterial and fungal rhizosphere microbiomes of 24 plant species, of which 16 belong to the Solanaceae family, under drought and well-watered conditions. At phylum level, drought induced consistent changes in the rhizosphere microbiome across species: an increase of Actinobacteria at the expense of Proteobacteria and an increase of Ascomycota at the expense of Olpidiomyota. Specific microbes that were most commonly drought-enriched in the rhizosphere of many plant species, but not in unplanted soil, were identified. In contrast, we also observed host species-specific patterns, such as the colonization of Solanaceae plants by Sphingobium bacteria. We next explored which microbial taxa correlate with drought resilience of the host plant and show that within the Solanaceae family, bacterial microbiome composition correlates with the underlying host plant phylogeny under well-watered conditions, but this correlation is weakened under drought. Lastly, we combined the rhizosphere microbiome data with untargeted liquid chromatography-mass spectrometry (LC-MS) of root metabolites to identify compounds that might recruit or deter specific microbes. We show that specific microbial taxa correlate in abundance with certain root metabolites, including compounds known to be involved in plant-microbe interactions or plant responses to drought.

rhizosphere, microbiome, drought, Solanaceae, root, metabolites

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65. Bacterial catabolic capacity for plant specialized metabolites underlies the bacterial fitness and the assemblage of root microbiota

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Plant specialized metabolites (PSMs), unique compounds produced by plants, play key roles in the interactions with root microbiota and current studies describe crucial functions of bacterial catabolic capacity for host-derived PSMs in these interactions. For instance, we previously showed that the genus *Arthrobacter* is a dominant bacterial taxon in tobacco roots, and these strains harbored a gene cluster for the catabolism of nicotine, a tobacco-specific PSM. This metabolic interaction, where root microbiota catabolizes host-derived PSMs, was also reported in soybean, tomato, sesame, and maize root microbiota, suggesting that these catabolism genes contribute to bacterial adaptation to their host roots. However, whether these bacterial catabolic capacities are indeed involved in the interaction with host plants remains unclear. In this study, we performed a combinational colonization assay using genetically manipulated *Arthrobacter* and tobacco mutants, both of which were impaired in nicotine catabolism and biosynthesis, respectively. This experiment showed that the nicotine catabolism capacity of the genus *Arthrobacter* improves their fitness in wild-type tobacco roots, but not in nicotine biosynthesis mutant. Using synthetic community (SynCom) approach, we further demonstrated the coordinately effects between PSMs and their bacterial catabolic capacity on the root microbial community structure. Collectively, our findings provided experimental evidence that the bacterial catabolic capacity for host-derived PSMs underlies the bacterial colonization phenotypes, as well as the assemblage of root microbiota.

Plant specialized metabolites, SynCom, Nicotine, microbiota

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66. Analysis of the α -tomatine degradation and the root colonization of *Sphingobium* sp. enriched in the tomato rhizosphere.

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Rhizosphere is defined as the soil area affected by plant roots. Plant metabolites secreted from the roots play important roles in plant-microbe interactions. Our previous research revealed that α -tomatine, a steroidal glycoalkaloid secreted from tomato (*Solanum lycopersicum*) roots, enriched Sphingomonadaceae, in particular the genus *Sphingobium* in the tomato rhizosphere. The enrichment of *Sphingobium* in tomato rhizosphere is commonly observed in field and pot cultivation experiments with three different Japanese field soils and in public database analyses. We isolated *Sphingobium* sp. RC1 from tomato roots, and it was found to induce root growth. RC1 degrades α -tomatine and uses it as a carbon source. This study aims to identify α -tomatine degradation enzymes of RC1 and to investigate the relationships between the α -tomatine catabolizing activity of RC1 and the colonization of tomato rhizosphere. Genome and transcriptome analysis of RC1 as well as the enzymatic characterization in vitro found several α -tomatine degradation enzymes. We then disrupted these genes involved in the sugar chain cleavage of α -tomatine and cultivated both RC1 wild-type and mutant strains in the presence of α -tomatine. The growth rate of the mutant strain was significantly lower than that of RC1 in the medium supplemented with α -tomatine. Furthermore, we evaluated the effect of α -tomatine as the sole carbon source in comparison between RC1 wild-type and mutant strains using minimum essential media. We are currently testing the chemotaxis of both RC1 wild-type and mutant strains to α -tomatine and colonization of these strains on tomato roots.

Rhizosphere, secondary metabolite, tomato, α -tomatine, *Sphingobium*

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67. The effects of structural diversity of flavones and flavonols on bacterial community in soybean rhizosphere

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Plants secrete a variety of plant-specialized metabolites from their roots into the rhizosphere, and these metabolites are involved in protection against abiotic stress and also in interactions with other organisms. Among flavonoids secreted by soybean roots, isoflavones are involved in nodulation process and shaping rhizosphere microbiota. However, the functions of flavones and flavonols in the soybean rhizosphere remain unknown. We cultivated 79 cultivars from world soybean core collections and conducted a metabolome-genome wide association study (mGWAS). The results of this study showed that several single nucleotide polymorphisms (SNPs) were significantly associated with the accumulation of flavones and flavonols, and these SNPs were found near the flavonoid 3'-hydroxylase (F3'H) gene. The accumulation of luteolin and quercetin, which have hydroxyl groups at the 3' -position in the B-ring, is decreased, whereas apigenin is relatively increased in the cultivars with the single nucleotide substitutions (Alternative type; ALT type) than in those without the substitution (Reference type;

REF type). This study aims to clarify the function of flavones and flavonols in the soybean rhizosphere using cultivars with differential flavone and flavonol accumulation in leaves. Ten cultivars of both REF and ALT types were selected and grown in the field, and the flavones and flavonols in leaves and roots were quantified. Two of these cultivars were further analyzed for rhizosphere microbiota and quantification of metabolites in the rhizosphere. The rhizosphere bacterial microbiome analysis at the genus level revealed that relative abundance varied in a cultivar-specific manner in several genera. Experiments with the application of flavones and flavonols to the field soil showed that they have different effects on the soil microbiota, respectively. We are currently working on the measurement of F₃H activity in REF and ALT type cultivars.

Plant-microbiome interactions / Plant secondary metabolite / Soybean rhizosphere / Flavonoid / Bacterial community / PGPB

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68. The Impact of Community Composition on Bacterial Volatile Organic Compounds Mediated Arabidopsis Growth Promotion

Gözde Merve Türksoy, Miroslav Berka, Kathrin Wippel, Anna Koprivova, Tonni Grube Andersen, Stanislav Kopriva

Volatile Organic Compounds (VOCs) serve as infochemicals in the interactions between plants and bacteria, play an important role in growth promotion, and protect against environmental stresses. While much attention has been directed towards understanding the growth-promoting effects of VOCs from individual bacterial strains, the broader implications of community-emitted VOCs on plant phenotype remain relatively unexplored. In our study, we assessed the impact of VOCs from root-derived synthetic bacterial community (SynCom) on Arabidopsis growth and root system architecture. Through profiling of the SynCom community composition and VOC emissions using Gas Chromatography-Mass Spectrometry (GC-MS), we revealed the intricate interplay among community composition and the resulting VOC profiles. Our findings show that plant growth promotion can indeed be mediated by VOCs from bacterial synthetic communities, albeit with distinct responses to individual community members. Notably, the removal of key species from the SynCom did not affect the growth promotion, suggesting that the modulation of plant responses by bacterial VOCs may stem from emergent properties of bacterial communities rather than the sum of actions of individual strains. Furthermore, our analysis reveals a stark contrast between the VOC profiles of single bacterial strains and synthetic communities, with the latter exhibiting a convergence towards a select few key compounds. This underscores the significant influence of bacterial interactions on VOC emissions within communities, likely driven by mutual metabolic modulation. Importantly, application of chemical VOCs could partly mimic the effect of the SynCom, validating the importance of VOCs in plant-bacteria interactions. In summary, our research shed light on the multifaceted roles of VOCs in plant-bacteria communication, emphasizing the need for understanding of community dynamics to harness the full potential of these bioactive compounds in agriculture and environmental management.

bioactive compounds, infochemicals, long distance signaling molecules, bacterial Volatile Organic Compounds

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69. Exploring the Role of Glycoside Hydrolase 25 in Fungal Lifestyle and Microbial Antagonism

Zarah Sorger, Sarah Daher, Priyamedha Sengupta, Katharina Eitzen, Eric Kemen, Gunther Döhlemann

Plants are colonized by a multitude of microorganisms, which interact not only with its host, but also display complex interaction networks within the microbial community. A scale-free high resolution network analysis of the phyllosphere microbiome of *A. thaliana* revealed that most of these interactions are antagonistic.

The pathogenic oomycete *Albugo laibachii* identified as a hub microbe in shaping the microbial community. The basidiomycete yeast *Moesziomyces bullatus* ex *Albugo* on *Arabidopsis* (short: MbA) inhibits *A. laibachii* and significantly reduces its virulence. RNA sequencing was conducted to discover genes involved in the inhibition of *A. laibachii*. This approach identified MbA candidate genes encoding putative secreted proteins including four putative glycoside hydrolases (GHs). The deletion of gene *g2490* (GH25) resulted in an almost complete loss of MbA antagonistic activity against *A. laibachii*.

This project aims to unravel the mechanisms by which GH25 functions and to explore its evolutionary conservation. GH25 of the pathogenic smut fungus *Ustilago maydis* is being explored, which shows 77% sequence similarity to MbA GH25 to address the evolutionary conservation of GH25 in the context of different fungal lifestyles. Preliminary data implies a comparable inhibition of *Albugo laibachii* by *U. maydis* GH25. The role of *U. maydis* GH25 in its pathogenicity will be further investigated.

To this point, the function of GH25 could not be linked to an activation of the plant immune system. Hence, the modulation of the microbial community by inhibition of its members might be a mode of action of GH25.

Indeed, one member of the bacterial community that is in close association with *A. laibachii* is inhibited by GH25. Moreover, this bacterium rescues the inhibition of *A. laibachii* by MbA and leads to GH25 upregulation. Ongoing experiments aim to elucidate if such multipartite antagonistic interactions are causal for the inhibition of *A. laibachii* by GH25.

Oomycetes, Phyllosphere, Beneficial Microorganisms, Antagonism, Plant-Microbe-Microbe Interactions

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70. Antimicrobial activity prediction sheds light on the evolution of fungal secreted effectors that manipulate plant host microbiota

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Plant microbiota comprise a broad diversity of prokaryotic and eukaryotic microorganisms that engage in fierce competition for host-derived nutrients. Several plant-associated fungi were recently shown to rely on the secretion of antimicrobial effector proteins to selectively antagonize microbial competitors to successfully colonize plant tissues. However, the occurrence and evolution of proteinaceous antimicrobials throughout the fungal tree of life

remains enigmatic. To address this knowledge gap, we developed AMAPEC, a software package dedicated to the annotation of candidate antimicrobial effector proteins in fungal secretomes based on their physicochemical properties. Since this predictor performs particularly well on fungal proteins, we used it to annotate catalogs of candidate antimicrobials in the secretomes of fungi that span a wide phylogeny as well as a large diversity of lifestyles. Our predictions revealed large numbers of candidate antimicrobial effectors, suggesting a broad occurrence of such proteins throughout the fungal kingdom. With this predictor in hand, we aim to address the hypothesis that antimicrobial effectors are ancient proteins of fungal ancestors that served in microbial competition in terrestrial and aquatic ecosystems prior to land plant evolution, and that are now crucial for microbiota manipulation during host colonization. Consistently, we predict antimicrobial activity in numerous effector families that are broadly conserved throughout the fungal kingdom. We validate the antimicrobial activity of particular effectors in vitro, and aim to demonstrate their role in microbiota manipulation during plant colonization. Additionally, we reconstruct the evolutionary histories of various fungal effectors with diverse functions to predict whether these evolved from ancestral proteins with antimicrobial activity.

fungi, effectors, microbiota, antagonism, antimicrobial, evolution

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71. Predicting Rhizosphere-Competence-Related Catabolic Gene Clusters with RhizoSMASH

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The capability of a soil microorganism to colonize the rhizosphere of a plant, known as rhizosphere competence, significantly influences the benefits a host plant could receive from their interaction. Approximately 20% of photosynthetically derived organic matter is distributed by plants into their rhizosphere in the form of root exudates. Hence, the ability of catabolizing the compounds in root exudates is one of most important determinants of rhizosphere competence. Despite the importance of catabolism in determining rhizosphere competence, the diversity of catabolic pathways, coupled with the functional diversification and redundancy of catabolic genes, have made it challenging to interpret catabolism from a (meta)genomic perspective. Thus, there is a demand for bioinformatic tools to analyze catabolic capacity in silico to advance our understanding of rhizosphere competence and the practical application of plant microbiomes.

In this study, we present rhizoSMASH, a new bioinformatic software package that leverages evidence from genomic synteny of enzyme-coding genes to identify and annotate rhizosphere-competence-related catabolic gene clusters (rCGCs) in bacterial genome sequences. We created 54 gene detection rules for rCGCs based on existing scientific research at 4 different evidence levels. These rCGCs cover degradation pathways for carbohydrates, organic acids, amino acids, biogenic amines, phytohormones, and aromatic metabolites. Our analysis of rhizoSMASH-predicted rCGCs across soil and rhizosphere bacterial genomes revealed extensive diversity and heterogeneity across taxa. Based on two published datasets with genome-sequenced bacterial strains whose rhizosphere competence were experimentally verified, we demonstrated that presence/absence profiles of rhizoSMASH-predicted rCGCs in bacterial genomes accurately predict rhizosphere competence. RhizoSMASH provides an extensible framework for studying catabolism in rhizosphere bacteria, offering new possibilities

in agriculture green development via designing of rhizosphere-accessible synthetic microbiomes or through rhizobacteria-targeted plant breeding.

rhizosphere, root exudates, catabolic gene cluster, machine learning

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72. Benzoxazinoids affect the growth of *Arabidopsis thaliana* through modulation of bacteria-bacteria interactions

Liza Rouyer, Claude Becker, Niklas Schandry

Benzoxazinoids are specialized metabolites secreted by grasses of the Poaceae family, such as maize, wheat and rye. In the soil, benzoxazinoids are converted to phenoxazines which can have a strong inhibitory effect on nearby plants and microorganisms. The conversion of benzoxazinoids to phenoxazines and other derivatives is driven by soil microorganisms, through mechanisms that are just starting to be investigated. Little is known on the role of benzoxazinoids and phenoxazines on the restructuring of the plant microbiome, and the consequent impact on plant growth and development.

We set out to assess the effect of benzoxazinoids and their phenoxazine derivatives on bacterial interactions, using BOA and APO as representative compounds. We screened over five thousand pairwise interactions between isolates of a bacterial collection from the *A. thaliana* and *L. japonicus* rhizosphere. We found that the presence of BOA and APO lead to changes in specific inhibitory bacterial interactions, with over a third of the observed interactions depending on the presence or absence of the tested compounds. In order to gain a better understanding of the mechanisms at play, we are conducting an in vitro transcriptomic analysis using bacterial pairs that display altered inhibitory interactions in the presence of benzoxazinoid compounds.

Next, we investigated how changes in bacterial interactions feedback plant growth. Using a gnotobiotic system, we found that BOA and APO can modulate the effect of bacterial inoculation on *A. thaliana* seedlings. We also found that the effect of these compounds on bacteria-bacteria interactions can modulate plant responses.

Our study shows that plant specialized metabolites can influence plant growth by altering pairwise interactions, thus modulating the composition root and rhizosphere communities.

benzoxazinoids, bacteria-bacteria interaction, rhizosphere

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73. Understanding the role of antimicrobial effector proteins secreted by the lichen-forming fungus *Peltigera rufescens*

Ciaran Kelly, Vivien Rosenthal, Hanna Rövenich, Björn Usadel, Bart Thomma

Symbiotic associations are found across the fungal kingdom, among which lichens are one of the most successful mutualistic fungal symbioses. By classical dual definition, lichens are highly complex interkingdom communities formed by two primary partners. While one is a lichen-forming fungus, termed the mycobiont, the other is a photosynthetic partner, called the photobiont that is typically an alga and/or cyanobacterium. Recent studies suggest basidiomycete yeasts and lichen-associated bacteria, which collectively form a microbiome,

as additional partners of lichen symbioses. Our group has demonstrated that pathogenic fungi utilize effector proteins with antimicrobial activity (AMPs) to modulate the microbiota in their surroundings to their advantage by suppressing microbial antagonists. We hypothesize that effector mediated microbiome manipulation is also fundamental to fungi with different lifestyles and therefore aim to understand the role of AMPs for lichen-forming fungi. In this study we focus on *Peltigera rufescens*, a cyanobacteria-harboring cyanolichen, for which a high-quality genome assembly was generated using Nanopore sequencing. Initial analyses of the mycobiont genome identified potential effectors with antimicrobial activity that might play a role in shaping the community structure of the lichen microbiota. For one of our candidates, produced via heterologous expression in *E. coli* and without known antimicrobial homologs, we observed antimicrobial activity in vitro on diverse bacteria and fungi. In future, we will investigate the role of *Peltigera rufescens* AMPs in shaping its microbiota composition and beyond focus on the functional characterization of *Peltigera rufescens* AMPs.

fungus, effector, lichen, antimicrobial, microbiota, symbiosis

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74. Unveiling the role of ROOMIE1 in root microbiome dynamics: insights from natural variation in *Lotus japonicus*

Johan Quilbé, Troels W. Mouritzen, Turgut Akyol, Yusdar Mustamin, Masaru Bamba, Shusei Sato and Stig Uggerhøj Andersen

Plant roots continually interact with a great diversity of soil microbes. However, the mechanisms governing how plants regulate bacterial colonization remain poorly understood. In this study, we employed a collection of *Lotus japonicus* accessions combined with a synthetic bacterial community, LjSPHERE, to identify host genes involved in rhizosphere colonization. Our analysis uncovered a significant signal in an uncharacterized gene, which we named ROOT MICROBIOME ESTABLISHMENT 1 (ROOMIE1). Disruption of ROOMIE1 led to drastic alterations in the root microbiome structure, both under inoculation with LjSPHERE and in native Japanese soil. Moreover, we identified two distinct ROOMIE1 haplotypes that correlated with different bacterial isolates and families. Both haplotypes were present in natural Japanese populations, and we observed intriguing distribution patterns among nearby individuals within the same location.

Root microbiota, Natural variation, Rhizobiome assembly, GWAS

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75. Deciphering bacterial functional assembly and root competence using Synthetic Communities

Florian Lamouche, Gijs Selten, Adrián Gómez Repollés, Zuzana Blahovska, Simon Kelly, Ronnie de Jonge, and Simona Radutoiu

Plant roots are home to a vast array of microorganisms, collectively known as microbiota. The assembly of host-associated root microbial communities is a complex process resulting from millions of interactions between plant roots and microbial cells. Historically, these communities have been primarily analyzed at the taxonomic level with very limited insight

through its functional assembly as a whole. In this study, we investigated the effect of the plant host and of the microbial environment on root community composition at the taxonomic and functional level. We established and used complex collections from three, taxonomically diverse plant hosts; *Arabidopsis thaliana*, *Hordeum vulgare* and *Lotus japonicus* grown in natural soils as microbial environment in unprecedentedly large reconstitution experiments. Detailed analyses of the established root communities enabled us to pinpoint how the original microbial environment, the host and the presence of different nutrition regimes impact the final root communities. Extensive dissection of the assembled root communities revealed key findings; i) a very limited number of functions are enriched across host-inoculum combinations, or by the different hosts; ii) individual isolates have different capacity to colonize and be retained as part of the root communities; iii) functions providing isolates with increased root colonization capacity can be identified using bacterial genetics tools. Our findings reveal both universal and host-specific root colonization mechanisms, highlighting new possibilities for robustly explore microbiome functionality at the level of individual isolates. This insight could be instrumental in developing sustainable root microbiomes or bioinoculants for agricultural applications.

Synthetic community, Functional composition, Root competence, Functional validation

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76. Plant disease resistance is determined to a large extent by ability to recruit a disease-suppressive rhizosphere microbiome

Shanshan Liu, Rong Li, Mohammadhossein Ravanbakhsh, George A. Kowalchuk

Agricultural productivity relies on the cultivation of disease-resistant plant varieties. Plant disease resistance is known to be a product of traits of both the plant and its associated microbiome, but the relative importance of the two is generally not known. We posit that the disease-resistance level of a plant is determined to a large degree by its ability to recruit a disease-resistant microbiome to the rhizosphere. Using banana resistance to *Fusarium* wilt as a model system, we examined the degree to which variety resistance was correlated with the plant's ability to stimulate specific bacterial and fungal taxa in the rhizosphere that are able to inhibit the pathogen. Furthermore, susceptible varieties could be rescued by inoculation to microbiomes recruited by resistant varieties, a result that could principally be attributed to bacterial transfer. We found that plant metabolic profiles in the rhizosphere were correlated with specific microbiome recruitment and identified potential key metabolites. Using synthetic communities (SynComs), consisting of strains of microbial taxa recruited by resistant versus susceptible varieties, we were able to demonstrate that resistant variety-associated SynComs were able to inhibit pathogen growth, with cross-kingdom (bacteria and fungi) SynComs showing the strongest effects. The levels inhibition by SynComs was further stimulated by the addition of specific key metabolites associated with resistant varieties. In total, our findings suggest that the ability to recruit pathogen-suppressive microbial taxa is an important component determining the level of pathogen resistance exhibited by plant varieties. This perspective opens up new avenues for improving plant health, in which both plant and associated microbial properties are considered.

Plant disease resistance, Rhizosphere microbiome, Bacteria, Fungi, Synthetic communities, Rhizosphere metabolites.

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77. Rhizobacteria's love language: decoding the basis of bacterial colonization patterns on roots

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To absorb essential nutrients and prevent the absorption of harmful compounds, plants rely on a specialized cell layer in the roots called the endodermis, which surrounds the central vascular system. As endodermal cells differentiate, the extracellular space between endodermal cells is sealed off by a lignin-based hydrophobic polymer, termed the Casparian strip. Nutrient quality and quantity vary along the root developmental axes, leading to preferential microbial colonization of specific root regions. Interestingly, most bacteria, including *Pseudomonas protegens* CHA0, tend to accumulate around the elongation zones and lateral root emergence sites, where Casparian strips are either not yet developed or transiently broken. Indeed, our research indicates that endodermal diffusion barriers not only regulate nutrient uptake but also restrict the leakage of bacterial attractants. By using the *Arabidopsis thaliana* myb36sgn3 mutant, which exhibits a strong endodermal diffusion barrier defect, we observed strongly enhanced CHA0 root colonization, attributed to both enhanced bacterial chemotaxis and proliferation. Metabolomic analysis of wild-type and mutant root exudates revealed elevated levels of amino acids in the latter, suggesting their potential role in enhancing bacterial colonization. We show that bacterial attraction to myb36sgn3 roots and wild-type lateral root emergence sites depends on chemotaxis and amino acid sensing, as CHA0 mutants deficient in these processes exhibited reduced colonization. To further validate that amino acids are indeed the underlying cause of bacterial colonization to lateral root emergence sites, we attempt to utilize CHA0 fluorescent reporter strains to visualize different bacterial metabolic states during root colonization. Our findings underscore the importance of tight control of nutrient provision to the rhizosphere, and provide insights into the biological basis of bacterial colonization patterns. This understanding could provide a foundation for advancing our knowledge of root-microbe interaction mechanisms and developing strategies to promote plant growth and health.

Pseudomonas , *Arabidopsis thaliana*, root colonization, amino acids

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78. Immune-stimulated plants change root exudation which is suppressed/ ignored by root associated bacteria

Charlotte Joller, Joelle Schlaepfer, Klaus Schlaepfer

Plants benefit from healthy microbiomes with increased resistance to biotic and abiotic stresses. A healthy microbiome requires tight regulation by the plant immune system. Otherwise, microbiomes become dysbiotic, for instance when commensal bacteria turn pathogenic on host plants impaired in immunity. Root exudates play a pivotal role in structuring root associated microbial communities. They contain both primary metabolites, providing easily available carbon, and secondary metabolites, including antimicrobials. Exudates are dynamically adapted to environmental conditions, for example in response to a pathogen infection. We study root exudate and microbiome dynamics after induction of pattern triggered

immunity (PTI), the first line of defense, via microbe- and damage-associated molecular patterns (MAMPs and DAMPs) in *Arabidopsis thaliana*. Under sterile conditions, MAMPs and DAMPs applied to the rhizosphere triggered a shift in root exudation. Amongst the compounds, which were reproducibly enhanced in exudation after immunity activation, we found defense-related compounds such as the glucosinolates. Interestingly, plants pre-colonized with a 9-member synthetic community (SynCom) of *A. thaliana* root-associated bacteria did no longer respond with a shift in root exudation or root tissue metabolite composition. Consistently, we found that root SynCom compositions did not differ between immune-stimulated and control plants. Our findings reveal dynamic root exudation in response to immune stimulation in absence of bacteria, which is not affecting and/or being suppressed by a root microbiota.

root exudates, plant immunity, SynCom, rhizosphere charlotte.joller@unibas.ch

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79. Soil diversity, impacted by the presence or absence of phages, affects plant-microbe communication in the rhizosphere

Genesisiska^{1,2*}, Tianci Zhao¹, Kira J. Tiedge¹, Joana Falcao Salles¹

The rhizosphere is a hotspot for interaction and communication between plants and microbes, shaped by exudates from both plants and microbes for chemical signaling. This study investigates the metabolic profile of root exudates from potato plants (*Solanum tuberosum*) after a drought and recovery period. In addition to the drought treatment, we grew the plants in experimental conditions with either high, intermediate (with and without phages), or low soil diversity. We discovered that soil diversity affects variation of the root exudates with a consistent trend from high to low diversity. Based on a partial least squares-discriminant analysis (PLSDA), we also explore that the root exudates generated by phage-free soils are clearly more similar to the exudates produced by the high diversity of soil than the exudates from phage-abundant soil. These findings indicate that phages in the rhizosphere may play a specific role in altering the microbiome population, particularly by selectively targeting and killing bacteria. Our research also revealed several interesting patterns of specific compounds being enriched in the potato rhizosphere under drought and recovery conditions and in regard to varying soil diversities. Moving forward, we will identify and investigate the most differential compounds, interpret their functionalities, and analyze the corresponding pathways. Furthermore, we will dig more into the role of phages for rhizosphere chemical diversity.

Drought; phages; potato; rhizosphere; root exudates; soil diversity

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80. Plants can select: Role of structural and chemical plant defense components as gatekeepers of microbial colonization

Uribe Acosta, M., Zhou, J., White F., Dong, L., Bouwmeester, H.J., Pieterse, C.M.J., Stringlis, I.A.

The resident soil bacterial communities include both pathogenic and beneficial bacteria, hence plants must select which colonize their roots. Plant structural (cutin, endodermal barriers, callose and lignin) and chemical (camalexin, indolic and aliphatic glucosinolates and

coumarins) defense components act as gatekeepers of microbial colonization. They have been thoroughly characterized by restricting pathogen colonization, however, their relative contribution to exudation and microbiome assembly remains largely uncharacterized. Here, we compared the exudation patterns and the microbiome composition of 16 *Arabidopsis thaliana* mutants affected in structural or chemical defense components. Moreover, to assess colonization, microbiome was studied in different compartments (bulk soil, rhizosphere, rhizoplane, endosphere) using 16S rRNA sequencing. We found that exudation patterns differed significantly between the mutants, with the endodermal and cutin mutants driving the changes. The compartmentalized microbiome analysis revealed a contrasting pattern between the chemical and structural mutants: The variation compared to the wild type communities increased towards the rhizosphere in the chemical mutants but in the opposite direction in the structural ones. While, compared to wild type plants, none of the structural mutants showed significant differences in the rhizosphere, in the endosphere one structural mutant showed the largest coefficient of determination: the endodermal barriers *myb36-2/sgn3-3*. This mutant also had the largest impact on the colonization dynamics of individual microbes. When comparing the bacterial classes in the rhizosphere and rhizoplane, chemical mutants showed a consistent enrichment of the bacterial class Bacteroidia. In the endosphere, patterns were clear only at finer taxonomic resolution, with both types of defense components impacting mainly families Oxalobacteraceae and Comamonadaceae. In current experiments we are evaluating the effect of these changes on plant health. Ultimately, we envision to unravel relevant plant mechanisms of microbiome recruitment.

Root defense, colonization, host-genotype effect, root microbiome, chemical defense components, structural defense components

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Session 3: Technological advances and translation

81. Common local and distinct variances. Supervised data integration in the plant holobiont.

Fred White, Roel van der Ploeg, Anna Heintz-Buschart, Lemeng Dong, Harro Bouwmeester, Johan Westerhuis, Age Smilde

Describing common local and distinct (CLD) variance structures is highly relevant for pathway detection in multi-omics studies of the plant holobiont. These (CLD) relate to patterns that can be found across (subsets of) the different omics/measurement types. We present our work in the development of statistical tools suited to uncover holobiont biological processes with an application in Tomato (data from; Abedini et al., in preparation). This dataset includes root transcriptomics, untargeted root extract LCMS and 16S metabarcoding of the rhizosphere. CLD discovery methods exist; Penalised exponential simultaneous component analysis (Song et al., 2019), here we present our extensions to the methodology to allow for the incorporation of a response as part of the model. In our application this response is a targeted metabolite which was not detected in the untargeted block. Through simulation studies and application to the example dataset we demonstrate how we can use the information in a single response to

uncover CLD patterns in the data - relating to biologically meaningful processes that might otherwise be undetected.

Multi-omics, Statistics, Data Integration, Data Fusion, Holobiont, Coexpression, Co-occurrence, Network

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82. Orochi: A modular and customisable pipeline for functional metagenome analysis

Lotte Pronk, Luisa M. Arias Giraldo, Mattias de Hollander, Belén Delgado-Martín, Victor J. Carrion, Anne Kupczok, Jos M. Raaijmakers, Marnix H. Medema

Metagenomics constitutes a prominent technology to study the functional potential of all organisms in a microbial community. Analysing the full potential of a metagenome dataset requires the use of many different analysis tools and methods, and can become a cumbersome and complicated process. Automated pipelines can speed up this process substantially, but may not contain every desired tool or analysis, and may be quickly outdated because of rapid advances in the field. We are developing an automated Snakemake pipeline, Orochi, that is modular, customisable, and provides output that is easy to work with and interpret. For co-assembly of multiple samples, our pipeline offers both supervised and unsupervised sample pooling, along with read normalization to accelerate the assembly process. Additionally, our pipeline retrieves high-quality MAGs by using the latest insights in binning and by including linkage of 16S genes to MAGs for higher taxonomic resolution. While assembly, binning, gene prediction, and functional annotation are part of most pipelines, we provide additional functionality. Firstly, many pipelines only focus on bacteria, but microbiomes may also contain eukaryotic microbes with possibly important ecological functions. By including eukaryote-identification at the contig level, and providing eukaryote-specific downstream steps, we aim to broaden the taxonomic scope of metagenome analyses. Secondly, finding biosynthetic gene clusters (BGCs) encoding the production of specialised metabolites in metagenomes can provide insights about the functions of the different organisms in microbial communities. antiSMASH is a popular tool for predicting BGCs, but with metagenomes it is slow, and the output is hard to interpret. Our pipeline includes a wrapper that optimizes antiSMASH for metagenomes and solves these problems. With these capabilities, Orochi provides a comprehensive and user-friendly suite of analyses, enabling users to uncover new insights into both the functional and taxonomic diversity of microbial communities.

metagenomics, bioinformatics, specialised metabolites, eukaryotes

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83. ASVNet: inferring microbes from amplicon sequencing data

Juan J. Sánchez-Gil and Ronnie de Jonge

Amplicon sequencing of the variable regions (V1-V9) of the 16S rRNA (rrn) gene is one of the most common techniques in microbiome research, and tools to analyze this type of data are abundant in the literature. However, analyzing amplicons in a whole community still imposes notable biological and technical difficulties. The main two approaches use fixed thresholds of

sequence similarity to collapse sequences into operational taxonomic units (OTUs, 97%) or amplicon sequence variants (ASVs, 99-100%). While ASVs avoid merging different species into a single cluster, it splits multiple copies of the same microbe into different clusters. On the other hand, OTUs at 97% similarity merge multiple organisms in a single unit, regardless of their functional or ecological similarity. Here we present ASVNet, a tool that leverages co-occurrence networks to infer the underlying organisms in a community as empirical OTUs, under the assumption that copies from the same genome will correlate strongly with each other across samples. Comparative tests reveal that ASVNet-derived eOTUs correspond more accurately to the original microbes and are more biologically meaningful than OTUs at any threshold. Our tool expands our ability to extract information from microbiome datasets by adding a more biological framework to amplicon sequencing problems.

Amplicon sequencing, 16S rRNA gene, ASV, OTU, microbial ecology, microbiomes, bioinformatics

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84. Challenges of microbiota preservation: the example of seeds

Perrine Portier, Cécile Dutrieux, Missimahou Oussou, Clara Maire, Steven Jagline, Isa Hollopp, Géraldine Taghouti

Plant microbiota are nowadays in the focus, being strategic for plant health, quality and resilience in a changing environment. However, their study can be impaired by technical limitations, making it still difficult to go beyond descriptive studies. One way to help scientist overcome these limitations could be to preserve these microbiota. Permitting for instance the a-posteriori isolation of interesting microorganisms, to use and reuse a set of particularly interesting community or to have access easily and rapidly to native or synthetic communities with specific features. However, the techniques used for isolated (and cultivable) microorganisms cannot be transposed directly to complex communities. Indeed, only a fraction of the microorganisms composing the complex communities are cultivable. Thus, the preservation of such communities will have an impact on the survival of the different components of these communities, leading to variations in the taxonomic composition. In turn, this may have an impact on the metabolic capacities of the community, possibly impairing its effect on plants. Within the consortium of the MICROBE project (<https://www.microbeproject.eu/>) our aim is to enhance the preservation of microbiota, in the objective to permit Biological Resource Centers, like CIRM-CFBP (<https://cirm-cfbp.fr>) and others, to one day offer the preservation of microbiota alongside individual strains. However, before getting there, a number of technological hurdles must be overcome. For instance, the best way to preserve the microbiota must be defined. But also, the best way to isolate the microbiota from its environment, the best way to prepare and process it, the best way to take it out from preservation or even how to disseminate it. The MICROBE project is focusing on four use-cases, one of them being the seeds in order to define the best modus operandi with the objective to translate the acquired experience to microbiota from other environments.

seed microbiota, preservation, technological hurdles

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85. Taxonomic resolution of different 16S rRNA variable regions varies strongly across plant-associated bacteria

Katarina Hrovat, Bas E Dutilh, Marnix H Medema, Chrats Melkonian

Plant-microbiome research plays a pivotal role in understanding the relationships between plants and their associated microbial communities, with implications for agriculture and ecosystem dynamics. Metabarcoding analysis on variable regions of the 16S ribosomal RNA (rRNA) gene remains the dominant technology to study microbiome diversity in this field. However, the choice of the targeted variable region might affect the outcome of the microbiome studies. In our in-silico analysis, we have evaluated whether the targeted variable region has an impact on taxonomic resolution in 16 plant-related microbial genera. Through a comparison of 16S rRNA gene variable regions with whole-genome data, our findings suggest that the V1-V3 region is generally a more suitable option than the widely used V3-V4 region for targeting microbiome analysis in plant-related genera. However, sole reliance on one region could introduce detection biases for specific genera. Thus, we are suggesting that while transitioning to full-length 16S rRNA gene and whole-genome sequencing for plant-microbiome analysis, the usage of genus-specific variable regions can achieve more precise taxonomic assignments. More broadly, our approach provides a blueprint to identify the most discriminating variable regions of the 16S rRNA gene for genus of interest.

Plant-microbiome, 16S metabarcoding analysis, Phylogenomics, Bioinformatics

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

86. Opposing effects of insect-herbivore-induced changes in the rhizosphere on plant-insect interactions in wild and cultivated cabbage

Kris A. de Kreek, Rieta Gols, Brigitte Noordijk, Ilva van Dam, Johannah de Zeeuw, Rob Nijhof, Karen J. Kloth, and Marcel Dicke

Plants can be severely damaged by herbivorous insects. In response, plants may recruit microorganisms through a belowground ‘cry for help’, leading to a change in the rhizosphere microbiome. Whether these recruited microorganisms help the plant in its defence against herbivorous insects remains to be assessed. Here, we investigated whether cabbage plants enhance their defence against insects when growing on soil previously conditioned by plants infested by insects. We did this through rhizosphere transfer in plant-soil feedback (PSF) experiments involving four caterpillar and three aphid species, two cabbage accessions (wild and white Brassica oleracea), and different soil types. Insect performance was assessed over time and defence gene expression was measured in the first two hours of insect feeding. Insect-herbivory-induced soil conditioning affected the performance of the caterpillar *Mamestra brassicae*. On wild cabbage, insect-induced PSF had a neutral to negative effect on *M. brassicae* performance and this effect became more pronounced over time. In contrast, insect-induced PSF was neutral to positive on *M. brassicae* on white cabbage and did not differ between soil types. Insect-herbivory-conditioned soil negatively primed jasmonic acid (JA) responsive genes in white cabbage, resulting in reduced upregulation of the JA pathway upon

M. brassicae feeding. This may explain the positive effect of insect-herbivory-induced soil conditioning on *M. brassicae* performance. Thus, cabbage rhizosphere changes upon insect attack may negatively affect plant defence and herbivore pest pressure in white cabbage while the opposite occurs in its wild relative. This research gives more insight into the role of the soil microbiome in plant defence against insects.

Rhizosphere; Plant-soil feedback; caterpillar; plant defence; Priming; Brassica oleracea

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Session 5: Plant microbial community ecology

87. The microvirome: Understanding phage-bacterial dynamics in the plant holobiont

Roitman, Sheila., Ashkenazy, Haim., Weigel, Detlef.

Eukaryotic organisms harbor large communities of microorganisms forming an holobiont, considered to be a single ecological and evolutionary unit. In recent years, bacterial community dynamics and their effect on the plant holobiont have been the subject of many studies. In spite of this, little is known regarding the role that bacteriophages play in shaping those bacterial communities. In my work I intend to set the basis for understanding the role of the microvirome in plant colonization and development, by studying *Arabidopsis thaliana* associated bacteria and phages, in laboratory and natural settings. Following a multilevel approach from isolates, to synthetic communities, to wild plants, I expect to gain a mechanistic understanding of the way phages affect plant-associated bacterial communities, deepening our basic understanding of the plant holobiont, and phage-host interactions in an oligotrophic environment. These findings can be projected to other significant plant-microbes systems, and be the foundation to design phage-based solutions to pest management in agriculture.

Bacteriophages, *Arabidopsis thaliana*, phage therapy, *Pseudomonas*

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88. Fungus-specific PCR primers for long read sequencing-based taxonomic analysis of plant microbiomes

Ting He, Koert Jansonius, Alison M. Reilly, Thomas Hackl and Kristina Haslinger

Endophytic fungi play a vital role in plant health and ecosystem functioning, yet their diversity and community structure remain understudied due to massive contamination with plant genomic and organellar DNA when extracting DNA from the holobiont. In this study, we developed and evaluated a set of fungus-specific PCR primers designed for nanopore long-read sequencing using R10.4.1 flowcells with Q20+ accuracy to analyze endophytic fungal communities within the medicinal plant *Vinca minor*. The primers were designed to target long regions of the ribosomal DNA (rDNA) locus, spanning both the small subunit (SSU) and the large subunit (LSU) or to specifically amplify endophytic fungi from total holobiont DNA. We validated the specificity and efficiency of the newly designed primers through in silico analysis and experimental evaluation using a laboratory synthetic community of fungal isolates, as well as extracted holobiont DNA from *Vinca minor*. Newly designed primers in this study exhibited high

specificity for fungal sequences and robust amplification across a diverse range of fungal taxa, both within the mock community and the holobiont DNA: with the best primer pairs, we produced fungal amplicons completely free of plant DNA. This demonstrates that the newly developed primers can be used to elucidate the diversity of endophytic fungi within *Vinca minor*, which might shed light on the potential roles of fungi in plant health and ecosystem dynamics. We are furthermore going to test these primers on various other plant species to test their broader applicability. Furthermore, the new primer design tool developed in this study should be universally applicable for the design of exclusive primers targeting certain phyla and offers a user-friendly and practical solution for researchers in microbiome research.

Plant endophytes; Plant DNA free amplification; Nanopore sequencing; fungus-specific primer; rRNA gene

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89. Transparent soils: a promising new technique to establish AM Fungi culture collections for accessible morphological assessment

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Arbuscular mycorrhizal fungi (AMF) form symbioses with over 70% of terrestrial plants and are key players in ecosystem services, plant health, and plant nutrient acquisition. While the interactions of plants and AMF are well-studied, the diversity of AMF mycelial network topology is under-represented. As the specific network topology (i.e., density, branching, interconnectedness, length, ...) may provide crucial insight in the network's resilience and efficiency of the fungal partner, this is undoubtedly an important research focus. An omnipresent challenge in AMF research is the culturing of this obligate biotroph under controlled experiment conditions. Although co-culturing both partners in natural soils may be closest to reality, the observation and analysis of the mycelial development in this setup is impossible. To investigate mycelial architecture, the preferred method is *in vitro* co-cultivation with (non-photosynthetic) root organ cultures. However, this is only possible for a small fraction of AMF species. By using a promising novel non-sterile technique, 'transparent soils', we aim to establish a culture collection method for AMF species found in natural soils. Similarly to bacterial and other fungal approaches, this allows simple, non-sterile culture establishment in transparent growth medium, morphological assessment, and selection for inoculation approaches of AMF. Transparent soils are not exclusively suitable for AMF-plant interaction studies but may be also applicable for studies of other plant-microbe interactions.

arbuscular mycorrhizal fungi, mycelial network, transparent soil, morphology, topology

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90. The effect of fungal-derived antimicrobial proteins is context- and condition-dependent

Gerlind Bauerecker, Edgar Chavarro Carrero, Hanna Rövenich, Bart Thomma

Plants actively recruit mutualists and beneficial endophytes through root exudates into their microbiota, allowing to alleviate abiotic stress and suppress pathogen invasion. Most soil-

borne pathogens infect the plant through the root, encountering the rhizosphere microbiota as a first layer of plant defense. The soil-borne, xylem-invading fungus *Verticillium dahliae* was shown to secrete antimicrobial effector proteins (AMPs) to manipulate the host microbiota, facilitating its invasion into the plant. Those AMPs show selective, yet distinct, activity spectra and can be antibacterial or antifungal or both. Individual expression patterns differ, and collectively they are not only expressed during host-colonization stages but also during soil-dwelling life stages of the fungus. Hence, AMPs potentially play a crucial role in facilitating fungal competition with various soil microbes. Considering that fungal evolution predates the emergence of land plants, and the likely intense competition with soil bacteria in early terrestrial systems in the quest for carbon, AMPs may initially have evolved as tools for microbial competition and might be conserved throughout the entire fungal kingdom. Here, we set out to investigate the effect of fungal-derived AMPs on communities of soil-derived microbes. The sensitivity of the individual bacteria to the AMPs was tested in different growth media. Subsequently, we assessed if the observed sensitivities change when other bacteria are present. Remarkably, we observe that certain bacteria, which are sensitive to an AMP when tested individually, become insensitive in the presence of certain other bacteria and vice versa. Collectively, our data suggest that antimicrobial activities of fungal-derived AMPs depend on environmental contexts.

antimicrobial, fungi, soil microbiome, activity spectrum, sensitivity

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91. Fungal diversity and community composition in rhizosphere soil of dragon fruit *Selenicereus megalanthus* differs among farms in the Ecuadorian Amazon region.

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"In Ecuador, yellow pitahaya is a tropical fruit widely accepted in national and international markets. However, the fruit is prone to many diseases incited by fungi, bacteria, viruses, and nematodes, leading to heavy losses in plant production, mainly caused by intensive agriculture, leaving a negative print on the soil microbiome. Since crucial information about the microbial diversity of yellow dragon fruit rhizosphere is missing, we aim to understand better the composition of fungal diversity and how it responds to different agricultural management. We characterized the diversity and community composition of fungal diversity in *S. megalanthus* crops using high-throughput sequencing of the ITS1 rDNA region. These were compared among eight farms near Palora in the Amazon region, with different management systems, including organic and conventional. DNA sequences were processed with the dada2 package implemented in R, and taxonomic assignments were made based on the UNITE database and were assigned to functional groups using FungiTraits. In total, 1572 fungal amplicon sequence variants (ASV) were identified, belonging to plant pathogens (e.g., *Fusarium*, *Phaeoacremonium*, *Corallomyces*), wood saprotrophs (e.g., *Humicola*, *Conlarium*, *Clonostachys*), litter saprotrophs (e.g., *Chaetosphaeria*, *Cladosporium*, *Nigrospora*), dung saprotrophs (e.g., *Apiosordaria*, *Enterocarpus*, *Preussia*), and several other functional groups. Significant differences in richness values were observed among the farms, and different patterns were observed among functional groups. In addition, the composition of soil fungal communities differed strongly among most farms.

Overall, all results indicate that most farms have distinct fungal communities, likely because of edaphic differences as well as differences in management practices, including soil manipulation, types of fertilization, and fungicides used."

dragon fruit, rhizosphere, fungal community, diversity, soil management

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92. Effect of metal-enrichment on bacterial and fungal communities associated with hyperaccumulating *Noccaea praecox*

Valentina Bočaj, Paula Pongrac, Matevž Likar

Cadmium, zinc, and lead hyperaccumulating plant *Noccaea praecox* (Brassicaceae) thrives at metal-enriched and non-polluted sites in Slovenia. To capture fungal and bacterial communities associated with the roots of this extraordinary plant, entire plants were collected with rhizosphere soil still attached at a non-polluted site in Lokovec (Slovenia) and at the metal-enriched site in Žerjav (Slovenia). Total genomic DNA was extracted from the rhizosphere using the DNeasy® PowerSoil® Pro Kit (Qiagen) and from roots using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma Aldrich). Shotgun metagenomic sequencing was conducted by MacroGen with the Illumina HiSeqX platform (2 × 150 pair-ends), using TruSeq DNA kit (Illumina). Altogether, 12 samples were sequenced: three repetitions for each rhizosphere and roots from both sites. Eight unique fungal and 262 unique bacterial Operational Taxonomic Units (OTUs) were found at the metal-enriched site, and six fungal and 211 unique bacterial OTUs at the non-polluted site, indicating the substantial influence of the plant species on fungal and bacterial communities associated with its roots. To assess the impact of the plant host on its root-associated microbiome in the metal-enriched site, we employed Differential Expression analysis of RNA-Seq (DESeq) alongside the Sloan neutral model. Fungal taxa abundance showed no statistically significant effects relative to the plant host, suggesting the dynamic of passive dispersal. By contrast, the bacterial community exhibited 37 genera above and 92 genera below the prediction based on neutral processes. This suggests some bacterial taxa may favor the plant host or environmental conditions, whereas genera above the prediction could be associated with active migration or negative interactions within the community.

hyperaccumulators, *Noccaea praecox*, root-associated microbiome, non-polluted site, metal-enriched site

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93. Identifying the core microbiome of legume plants under phosphorus stress

Justin D. Stewart, Malin Klein, Sébastien Jaupitre, Vasilis Kokkoris, E. Toby Kiers, James T. Weedon

Phosphorus (P) is one of the most limiting nutrients in crop fields globally. While plants have evolved remarkably diverse root architectures that can significantly vary in response to nutrient conditions, growth of plants in many situations remains phosphorus limited. There is a potential to improve the P uptake efficiency of plants by exploiting the genetic and functional diversity of root associated microbes. Still, directed application of the rhizosphere microbiome

to alleviate phosphorus deficiency relies on identifying which microbes are recruited from the soil to the rhizosphere under phosphorus limitation. Until now, identifying a core group of microbes recruited under phosphorus stress has been difficult, limited by: (i) a lack of standardization across studies (e.g. different soil chemistry, microbiomes), (ii), a lack of comparison of microbiomes across plant species, and (iii) a lack of quantified uncertainty for each member of the core-microbiome. We grew 22 plant species (including 15 Fabaceae) under high and low phosphorus stress and characterized the microbial communities using marker gene sequencing. We found that host plant identity explained ~45% of variation in bacterial richness with on average 115 ASVs recruited under phosphorus stress using linear mixed-effects models. Still this varied significantly, with *B. oleracea* losing > ~1000 ASVs and *L. culinaris* recruiting ~1000 ASVs. Across all the legumes we studied, bacteria associated with P stress mostly come from classes Alphaproteobacteria, Actinobacteria, and Acidimicrobiia. This result was robust to a range of choices in abundance and occupancy thresholds.

phosphorus stress, core microbiome, phylogeny, uncertainty, bacteria

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94. Evolution soil microbial biodiversity under green cover crops management

Sandra Tienda, Belén Delgado-Martín, Ana Lia Gayán, José Antonio Gutiérrez-Barranquero, Javier Peris, Eva Arrebola, Francisco M. Cazorla and Víctor Carrión

This research has been carried out within the international LIVINGRO® project (Syngenta), and is based on the application of ecological best management practices such as the multifunctional inter-row cover crop in different cropping systems, with the objective to optimize environmental sustainability and reducing the negative impact of intensive conventional agriculture. The study was carried out on selected several plots of stone fruit in different locations in Spain. Soil samples were collected during three years and the effect the inter-row vegetation cover crop can have on microbial biodiversity was studied. The alpha- and beta-diversity analysis was performed at the Prokaryotic (mainly bacteria) and Eukaryotic (fungi) level, and putative microbial indicators of specific vegetation cover management soil were proposed. The results indicate that the evolution of alpha diversity values decreases at the bacterial level and increases at the fungal level over time, while no differences were observed between the ecological and control treatments. The beta diversity results show that in general there is compositional differentiation of populations by sampling time, both at the prokaryotic and eukaryotic levels. And we observed that in each sampling the vegetation cover has an effect on the composition of the bacterial and fungal populations, enriching specific microbial groups that could potentially have a beneficial role for soil and plants.

fruit trees, sustainable agriculture, soil health, microbial markers

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95. EXPLORING SOIL MICROBIOME BIODIVERSITY OF AVOCADO CROPS IN SOUTHERN SPAIN UNDER CONVENTIONAL AND ORGANIC MANagements

Blanca Ruiz-Muñoz, Sandra Tienda, Francisco M. Cazorla and José A. Gutiérrez-Barranquero

Avocado (*Persea americana* Mill.) is a commercially important subtropical fruit with renowned health benefits. The Axarquía region in Southern Spain, located at the province of Malaga, stands as Europe's primary producer of this valuable crop due to its favorable subtropical climate. However, the region faces significant challenges, predominantly from fungal pathogens, threatening both crop survival and yield. Organic agriculture and sustainable soil pathogen control strive to reduce the use of chemical fertilizers and pesticides in order to improve ecosystem health. Despite the critical role that microbial communities play in agricultural output, we still have a limited understanding of how microbial diversity responds to organic and conventional farming.

This study aims to compare the long-term effects of organic and conventional production systems on avocado plant in soil microbial communities. We selected two orchards in the Axarquía region that have been under these two types of managements for over 20 years. Rhizosphere samples were collected over the course of a year season, at four different sampling times that corresponds to the avocado plant's main phenological stages. We assessed the microbial community structure using amplicon sequencing of the 16S and ITS rRNA genes. At the prokaryotic level, the organic treatment showed a trend towards higher alpha diversity. According to beta diversity, the composition of bacterial and fungal microbial populations differed between organic and conventional managements. Interestingly, the organic management resulted in a specific recruitment of potential beneficial microbial genera associated with biological control or nutrient cycling, among others. This study underscores the influence of agricultural management practices on avocado microbiome and gives novel insights to improve the sustainability of agro-ecosystems through the use of beneficial microorganisms.

Organic agriculture, conventional management, ecosystem health, microbial diversity

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96. Assessing microbiome population dynamics in the phyllosphere using wild-type isogenic standardized hybrid (WISH)-tags

Benjamin B. J. Daniel, María Victoria Berlanga-Clavero, Yves Steiger, Anna Sintsova, Shinichi Sunagawa, Wolf-Dietrich Hardt & Julia A. Vorholt

Microbiomes are ubiquitous and play important roles in ecosystems. They provide symbionts with vital nutrients, confer colonization resistance to host organisms, and are key players in element cycling from the micro to the global scale. In general, microbiomes of the same habitat form relatively stable communities over time regarding species composition. However, the underlying population dynamics within species are largely unknown, as are the rules for community assembly that could help build synthetic microbiomes with desired functions.

To address this gap, we have developed a genomic barcoding system capable of discriminating bacterial populations, referred to as WISH tags (for Wild-type Isogenic Standardized Hybrid-tags). We experimentally validated 62 WISH-tags showing their specificity in quantifying isogenic strains across seven orders of magnitude without interference from the host genomic background. Using WISH-tags in the context of *Arabidopsis thaliana* phyllosphere, we investigated intrastain priority effects of *Sphingomonas* Leaf257, a member of a genus that is ubiquitous in the phyllosphere. We found that late-introduced populations are accommodated proportionally to their presence at the inoculation timepoint. This observation suggests that

early and late-arriving strains have equal chances to colonize new habitats and exemplifies how WISH-tags can be used to decipher population dynamics within microbiomes.

Microbiomes, population dynamics, bacterial tracing, colonization resistance, *Arabidopsis thaliana*, priority effects

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97. Ecological characterization of the rhizosphere microbiome of wheat grown under drought conditions in successive cultivation

Adele Pioppi, Caja Dinesen, Xinming Xu, Ákos T. Kovács

Current efforts to ensure sustainable crop production include characterization of plant rhizosphere microbiomes. In fact, different microbial groups influence plants by inducing or helping to relieve stress. Further, microbes respond to plant-derived signals, which may result in abundance shifts of selected taxonomic groups. Successive cultivation of plants under different treatments can enhance such alterations in the microbiome composition via repeated reinoculation of the rhizosphere microbiome of select plants. In this work, we hypothesized that applying drought stress to wheat during successive cultivation highlights key bacterial groups that contribute to plant stress resilience. Our experimental setup included a randomized selection lineage under non-stressed conditions, a selection lineage for robust drought-resilience, and a selection lineage for poor drought-resilience (susceptible). Additionally, these lineages were either supplied with an 84-member wheat-derived bacterial community in the first cycle, or non-inoculated with additional strains. 16S rRNA amplicon sequencing was performed on the rhizosphere of plants after the last cycle to identify bacterial groups with a different relative abundance between the treatments, particularly differentiating the treatments involving drought compared to well-watered conditions. Within both resilience-selected and susceptibility-selected lineages there was considerable variation in composition. Still, several bacterial groups could be identified that were connected to plant stress responses across treatments. The characterization of key microbial players in the rhizosphere represents a relevant starting point for further in planta studies under drought conditions, and highlights the value of performing successive cultivation in plant microbiome studies.

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98. Exploring the microbiome with a special focus on AMF and N-fixing bacteria associated with the rizosphere of the genus *Inga* in the pristine Ecuadorian Amazon

Arévalo-Granda V, Hickey-Darquea A, Quiñonez CF, Jiménez-Hurtado D, Prado-Vivar B, Zapata S, Duchicela J, van 't Hof P.

This study explored the microbiome composition in the rhizosphere of *Inga* seedlings in two neighbouring forest ecosystems in the undisturbed Amazon rainforest at the Tiputini Biodiversity Station in Ecuador. In terra firme plots, which were situated higher up and therefore typically outside of the influence of river floods, and in várzea plots, the lower forest located near the riverbanks and therefore seasonally flooded, tree seedlings of the genus *Inga* were

randomly collected and measured, and the rhizosphere soils surrounding the root systems was collected. *Inga* sp. seedlings collected in terra firme showed a lower shoot-to-root ratio compared to várzea seedlings, suggesting that *Inga* seedlings which germinated in várzea soils could invest more resources in vegetative growth with shorter roots. Physical-chemical properties of the soil samples indicated higher proportions of N, Mo, and V in terra firme soils, whereas várzea soils present higher concentrations of all other macro- and micronutrients, which confirmed the nutrient deposition effect of seasonal flooding by the nearby river. Bioinformatic analysis using ITS metabarcoding was performed to calculate the alpha and beta diversity, species taxonomy and the differential abundance of fungi and arbuscular mycorrhizal fungi using QIIME2. The fungal community represented 75% of the total ITS ASVs, and although present in all samples, the subphylum Glomeromycotina represented 1.42% of all ITS ASVs with annotations to 13 distinct families, including Glomeraceae (72,23%), Gigasporaceae (0,57%), Acaulosporaceae (0,49%). Full-length 16S rRNA gene amplicon analysis of root nodules of members of the genus *Inga* using MinION™ nanopore sequencing conferred the presence of N-fixing bacterial consortia. Results of this study indicate that AMF surround the rhizosphere of *Inga* seedlings in relatively low proportions compared to other fungal groups, but are present with N-fixing bacteria in root nodules in both terra firme and várzea Neotropical ecosystems.

microbiome, *Inga* (Fabaceae), arbuscular mycorrhizal fungi (AMF), Glomeromycotina, N-fixing bacteria, Amazon rainforest, Ecuador

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99. The tomato fungal partners: Mycobiome composition associated with endemic and introduced tomato species at the Galapagos Islands.

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The endemic Galapagos tomato species *Solanum galapagense* (GAL) and *S. cheesmaniae* (CHS) have evolved to survive under harsh environmental circumstances in arid, saline, and/or volcanic soils. Although the genetic background responsible for the high tolerance to these stresses has been extensively studied, surprisingly little is known on the taxonomic and functional microbial diversity associated with the endemic Galapagos tomatoes. The plant-associated mycobiome can influence plant growth, nutrient acquisition and cycling, and protection against pathogens. In this study, we explored the mycobiome composition of both tomato endemic GAL and CHS species, the domesticated species *S. lycopersicum* var. (LYC) and the invasive native species *S. pimpinellifolium* (PIM) collected on the islands Isabela, Santa Cruz, and San Cristóbal. ITS metabarcoding was used to analyze the fungal communities present in the phyllosphere and rhizosphere of the 4 tomato species. Our analysis showed that the rhizosphere samples were primarily dominated by Aspergillaceae and Plectosphaerellaceae, whereas not assigned families, Pleosporaceae and Periconiaceae are the most abundant in the tomato phyllosphere. These diverse families include plant pathogenic genera (e.g. *Aspergillus*), saprophytes, and members that produce secondary metabolites with antibiotic activities (e.g. *Penicillium*). The Shannon index in the LYC rhizosphere was significantly higher than in the other three tomatoes. Moreover, the β -diversity showed that fungal communities clustered together within each tomato species. In the GAL and CHS phyllosphere, α -diversity was significantly lower than in LYC and PIM. α -diversity showed that mycobiome was not significantly different between GAL populations collected from Isabela and

Floreana islands. Our study reveals insights into how the Galápagos tomatoes recruit fungi in their rhizosphere, and phyllosphere. Our study provides a first insight into the fungal community structure associated to endemic and introduced tomato species at the Galápagos archipelago, highlighting the influence of biogeography on mycobiome composition, and the selective recruitment of fungi associated with tomato growth and adaptation in the archipelago environment.

Endemic tomatoes, mycobiome, phyllosphere, rizosphere, Galápagos Islands.

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100. The Plant Coumarin Scopoletin Modulates Natural Product Biosynthesis by Endophytic Streptomyces

Emtinan Diab, Chao Du, Wendy Tigani, Somayah S. Elsayed, Gilles P. van Wezel

Microorganisms living in symbiosis with plants can provide beneficial functions to their host via production of specialized metabolites, yet, regulation of their biosynthesis in response to plant signals is understudied. Coumarins are a well-described class of plant metabolites. These compounds play multiple roles to support plant fitness including iron solubilization in alkaline soil and antimicrobial activity against phytopathogens (Stringlis et al., 2019). Several studies describe the roles of coumarins as agents controlling microbiome recruitment (Voges et al., 2019); however, the roles of coumarins as chemical signals perceived by plant-associated microbes is not as thoroughly researched. We sought to investigate the effect of scopoletin on natural product (NP) biosynthesis by endophytic Streptomyces, a genus of Actinobacteria often referred to as Nature's medicine makers. We discovered that scopoletin can alter the production of bioactive compounds by the endophyte Streptomyces sp. ATMOS53 isolated from *Arabidopsis thaliana* roots. Using multi-omics approaches of metabolomics, proteomics, and genomics, we showed that scopoletin interferes with the glycosylation of anthracyclines, which are clinical anticancer drugs produced by type II polyketide synthases (PKS). Scopoletin interrupted the glycosylation steps of the polyketide backbone, as shown by the accumulation of non-glycosylated intermediates and decreased production of the final glycosylated product. The glycosylation profiles of anthracyclines have significant effect on their cytotoxicity (Křen and Řezanka, 2008; Gui et al., 2018). Conversely, scopoletin stimulated the production of bohemamines, a class of bacterial alkaloids synthesized by non-ribosomal peptide synthases (NRPS). This was correlated to enhanced levels of the biosynthetic enzymes. Taken together, our results demonstrate that scopoletin has multiple and divergent effects on the expression of bioactive compounds in endophytic Streptomyces sp. ATMOS53. Our work highlights the importance of accounting the ecological chemistry of plant-microbe interaction studies to fully unravel the capabilities of microbes considered to be used as bio-control agents.

Coumarin, scopoletin, Streptomyces, natural products, anthracyclines, bohemamines

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101. Forest connectivity drives the litterosphere: fungal community and functionality under Atlantic Forest restoration

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Tropical forest restoration is critical to mitigating biodiversity loss and ecosystem degradation. It is known that restoring forests in a fragmented landscape may hinder the recolonization of aboveground biodiversity. However, effects on restoring belowground biodiversity and soil functioning are essential for nutrient cycling and maintaining forest productivity but remain poorly explored. Here, we study the role of forest connectivity in restoring fungal communities and their functionality in the litter layer of the Brazilian Atlantic Forest by examining differently aged restoration sites and original forest patches. We tested these effects in a chronosequence forest restoration ranging from abandoned pasture to 18-55 years old secondary forest to pristine primary forest. We quantified how forest age and connectivity to the original forest influenced tree diversity and richness in the restored forests, chemical litter characteristics, C and N isotope signatures of the litter, and the composition of litter fungi and fungal functional guilds. Our findings revealed that forest connectivity influenced fungal guilds more than forest age, promoting saprotrophs, and endophytes and suppressing plant pathogens, especially in the early stages of forest restoration. Increasing forest connectivity corresponded with an increase of tree diversity, and litter N and P content and had higher correlations with fungal guilds. Furthermore, early secondary forests with lower connectivity were less associated with specialized fungi than those with higher connectivity, but this trend was reversed in the later stages of restoration. Interestingly, we observed a rise in ^{15}N and a decline in ^{13}C concentration in older forest litter. These findings underscore that forest connectivity matters and is critical for below-ground communities by affecting the abundance of fungal functional guilds.

Forest chronosequence; Ecosystem services; Biological indicator; Fungi sequencing; Landscape connectivity; Fungal guilds

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102. Diversity Matters: rhizosphere microbiome influence on wheat protection against soil-borne pathogens

Caroline Sayuri Nishisaka, João Paulo Ventura, Hélio Danilo Quevedo, Fernando Dini Andreote, Rodrigo Mendes

The rhizosphere microbiome is crucial for plant growth and development, including defense against soil-borne pathogens. Despite numerous biological products entering the global market, our understanding of how these inoculants interact with the rhizosphere soil microbiome diversity influencing plant protection remains limited. This study aimed to evaluate the impact of rhizosphere microbiome diversity on wheat plants inoculated with the beneficial *Pseudomonas inefficax* strain CMAA1741 and exposed to the soil-borne pathogen *Bipolaris sorokiniana*. Using the dilution-to-extinction approach, a bioassay was conducted with natural soil three different dilutions of natural soil mixed with autoclaved soil and autoclaved soil along with treatments containing plants inoculated with *P. inefficax* and/or *B. sorokiniana*, as well as control plants (water-treated) and bulk soils.

Plants inoculated only with *P. inefficax* exhibited increased height and root dry mass. As expected, the disease severity index was higher in plants inoculated with the pathogen in soils with lower microbial diversity. Biocontrol efficacy by the *P. inefficax* was also higher in soils with lower microbial diversity. Beta-diversity analyses indicated significant changes in bacterial and fungal communities' composition when *P. inefficax* and/or *B. sorokiniana* were inoculated. Furthermore, a few bacterial and fungal taxa were detected only when *P. inefficax* was inoculated, including members of *Flavisolibacter*, *Chthoniobacter*, *Massilia*, and *Streptomyces* genus. Inoculation only with *P. inefficax* led to an increase in co-occurrence network modularity and when the pathogen is present, in plants inoculated with *P. inefficax*, there was a decrease in network modularity and number of nodes and an increase in the number of edges.

In low microbial soil diversity, when plants are more susceptible to soil-borne pathogens, the inoculation of beneficial bacteria changes the structures of bacterial and fungal communities, preventing pathogen invasion of the rhizosphere.

rhizosphere microbiome, dilution-to-extinction, *Bipolaris sorokiniana*, *Pseudomonas inefficax*

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103. Host-specific arbuscular mycorrhizal communities are maintained across varying plant communities

Cynthia Albracht, François Buscot, Anna Heintz-Buschart

Arbuscular mycorrhizal fungi (AMF) are ubiquitous plant symbionts present in the majority of terrestrial plants. While we are aware of a variety of exchanges between AMF and the host plant, certain factors of this plant-fungi-symbiosis are still unclear. One aspect repeatedly discussed is the host specificity of AMF. Plants have been shown to accumulate a specific community of AMF, but how consistent such communities are under different conditions remains unknown.

To tackle this knowledge gap, we made use of the experimental grasslands in the long-term biodiversity platform The Jena Experiment. The experimental grasslands span a plant species and functional diversity gradient (1 to 60 grassland species; 1 to 4 functional groups) in randomized combinations. We sampled individuals of 16 resident plant species in their monocultures and across the diversity gradient. To address the consistency in host-specific AMF communities, we performed amplicon sequencing (SSU) on root, rhizosphere, and bulk soil samples and estimated mycorrhizal colonization intensity through microscopy and qPCR of roots. We further related these data to plant (root) traits and biomass.

We identified vicinity to the host as main determinant of specific AMF community upkeep. Bulk soil and rhizosphere, which shared AMF, tended towards an increasing AMF richness with plant diversity. Contrarily, AMF diversity showed stronger unevenness in more diverse grasslands. AMF community structures deviated more the further away they were from their host plant, i.e. community composition in roots was more consistent than in rhizospheres and bulk soils. The retention of host-specific AMF communities seems to further be driven by functional identity of the host and the surrounding plant community. In short, host-specific arbuscular mycorrhizal communities, especially within roots, are consistent across varying plant communities.

arbuscular mycorrhizal fungi, host-specificity, experimental grasslands

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104. The co-occurrence of soil protists and prokaryotes is affected by wheat variety and rhizocompartment

Christine Lorenzen Elberg, Rumakanta Sapkota, Athanasios Zervas, Anne Winding

Wheat (*Triticum aestivum* L.) is an important agricultural crop grown worldwide. Protist communities and their interaction with prokaryotes in soil and rhizosphere have been shown to influence crop performance, yet our understanding of the diversity, the communities, and the driving factors that govern them are limited. The significance of the wheat rhizosphere on protist communities were studied in a greenhouse pot experiment growing four wheat varieties to early flag leaf stage. The rhizosphere was divided into three rhizocompartments (rhizoplane, rhizosphere, and bulk soil) and DNA was extracted. Protist abundance was determined using qPCR while 18S rRNA gene metabarcoding described the protist community composition. The co-occurrence with prokaryotes was assessed using data of prokaryotic community composition by 16S rRNA gene metabarcoding.

We found significant effects of the rhizocompartment, wheat varieties, and plant heights on protist community structure and abundance. Specifically, protist richness was significantly higher further away from root surface. Total protist abundance was higher in the Rembrandt wheat variety while amoebae abundance was lower in the Kvium wheat variety. Differential abundance and Random Forest analysis revealed that Colpodea was relatively more abundant in the rhizosphere than in bulk soil, and Filosa-Sarcomonadea were relatively more abundant in the rhizoplane than further away from the root. A co-occurrence network analysis of prokaryotes and protists showed a more intricate network with a higher number of nodes further away from the root. Overall, our results show that rhizocompartments and wheat variety drive protist community compositions, which is consistent with the drivers of prokaryotic communities and demonstrates the interconnectivity of protist-prokaryotic interactions in rhizosphere.

rhizosphere, rhizoplane, 18S rRNA, qPCR, *Triticum aestivum*, bacteria

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105. Evidence of host selection on the epiphytic phyllosphere communities in a physiologically enigmatic medicinal tree

Anya S Noble, Charles K Lee

Leptospermum scoparium (mānuka) is an indigenous New Zealand tea tree widely known for the unique antibacterial properties of its honey. However, the host physiological traits associated with these properties exhibit variation that remains unexplained despite decades of research on host genetics, endophytes, and environmental factors.

A preliminary investigation of the bacterial epiphytic communities in the mānuka phyllosphere revealed a dominant and ubiquitous core microbiome (100% prevalence at the OTU level) across geographically distant and environmentally diverse populations of mānuka. In accordance with the core microbiome concept, this strong spatial association suggests that some phyllosphere taxa may have an influential role in host physiology. Thus, we raised the

question: do host-microbiome interactions in the phyllosphere drive mānuka physiological heterogeneity?

Using natural populations of mānuka, we tested mechanisms of community assembly congruent with those of a microbiome that exhibits strong host interactions. Specifically, we hypothesised that community assembly in the mānuka phyllosphere microbiome is primarily driven by direct host selection.

Using a combination of spatial and temporal sampling designs, we demonstrated that host species identity is the strongest driver of community assembly in the mānuka phyllosphere, relative to abiotic factors and stochastic processes. Furthermore, using pairwise comparisons of mānuka with kānaka, an ecologically and morphologically indistinguishable yet phylogenetically distant plant, we demonstrated that the host factors that drive the specificity of the mānuka phyllosphere microbiome are independent of morphological and ecological traits. Our results thus hint at direct host selection mechanisms (e.g., via chemicals).

Our work has demonstrated the potential of mānuka as an intriguing model system for advancing a mechanistic understanding of direct host selection and host-microbiome interactions in the phyllosphere of perennial tree species. We also shed new light on the processes of phyllosphere community assembly and provide several important considerations for future research on phyllosphere microbial ecology.

phyllosphere microbiome, community assembly, *Leptospermum scoparium*

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106. Tillage impacts soil protist community

Julie Egelund Andersen, Athanasios Zervas, Rumakanta Sapkota, Anne Winding

Protists are unicellular eukaryotes that are highly diverse and often overlooked displaying a vast array of functional traits. Their assembly and distribution are mainly structured by deterministic environmental processes such as soil moisture, pH, and nutrient availability rather than through stochastic events and passive dispersal. Nevertheless, the main drivers of the protist community structure remain poorly understood.

The impact of agricultural practices i.e. conventional tillage vs. no-tillage, pig slurry vs. chemical fertilizers, and three different nitrification inhibitors on soil protist diversity were studied in a field experiment in 2021. In soil grown with winter wheat (*Triticum aestivum*) at Foulum, Denmark, the tillage and fertilizer regimes were varied. The genetic diversity of the protist community in 60 soil samples was profiled by metabarcoding using protist-specific primers targeting the V9 region of the 18S rRNA gene. To understand protists as organisms with different properties, we assigned trait-based functional diversity to our taxonomic data.

Diverse protist community structures within the phyla of Cercozoa, Amoebozoa, and Ciliophora with a dominance of Cercozoa were found. Using PERMANOVA tests tillage and fertilizer regimes were found to be vital factors in shaping the protist community at the genus level. Organically fertilized soil exhibited a distinct protist community composition compared to chemically fertilized soil. The trait-based approach revealed the functional protist diversity to be affected mainly by the tillage regime. The relative abundance of endoparasitic protists was

higher in no-tillage soils compared to conventional tillage. Consequently, an increase in the relative abundance of free-living protist consumers was observed in conventionally tilled soils.

These results showed the significance of agricultural practices on the trait-based functional protist diversity. Knowledge that may be helpful for farmers when deciding on tillage and fertilizer regimes.

microbiome, metabarcoding, drivers, fertilizer, trait, endoparasite

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107. Multi-generation transmission of plant microbiota: learning from naturally assembled communities and bioinoculants

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The holobiont theory posits that some core microbial taxa are functionally significant for the plant and form long-lasting interactions that involve vertical transmission through seeds. Still, few studies aimed to assess the contribution of vertically transmitted taxa to the assembly of seed and plant microbiota during its entire life cycle. In this study, we characterized the transmission of seed microbiota for different plant species (*Arabidopsis thaliana* and *Phaseolus vulgaris*) for multiple generations (5 and 3, respectively) under climatic chamber conditions. Using metabarcoding (*gyrB* for bacteria and ITS1 for fungi), we found that a small fraction of the seed microbiota was faithfully transmitted between generation (2 to 7 ASVs) across all parental lines, with more stable transmission for fungi than bacteria. Interestingly, these few taxa were extremely abundant in seeds (>50% relative abundance) and in aboveground compartments of adult plants (stem, leaves, >20%). The inherited taxa were affiliated to *Brevundimonas*, *Pantoea agglomerans*, *Pseudomonas putida* for bacteria and *Cladosporium* sp, *Apotrichium* sp, *Mortierella elongate*, *Giberella* sp for fungi. Next, we aimed to assess the impact of bioinoculant inoculation on parental plants (reproductive stage) on seed microbiota and the transmission of seed-borne taxa to the next generation. We used the commercial product Cerall (Koppert) containing a *Pseudomonas chlororaphis* strain used for the biocontrol of fungal pathogens transmitted by seeds on 4 different genotypes and 2 field sites. The biocontrol strain was rarely detected on seeds but had a significant effect on seed microbiota assembly and seedling growth for 2 genotypes. These results show that vertical transmission of seed taxa between generation is observed only for small fraction of seed microbiota and that field inoculation of biocontrol strains can have lasting effects on seed microbiota and seedling phenotype even in absence of a successful colonization.

microbiota assembly, vertical transmission, seed microbiota, microbial invasion

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108. Contribution of floral transmission to seed microbiota assembly in watermelon

Gillian E. Bergmann, Kacie Lui, Carina Lopez, Alexander Velasco, Coralie Marais, Matthieu Barret, Marie Simonin, Rachel L. Vannette, Johan H.J. Leveau

Seeds typically carry bacterial communities that parent plants transmit to their offspring and that can impact seed germination and seedling development. Some members of these seed-associated communities are acquired via the floral route, which involves movement from the flower stigma to the developing seed. Evidence for the importance of the floral pathway in the assembly of seed microbiota is scarce and mostly limited to studies with seed-borne pathogens. Here, we investigated the contribution of the floral transmission route to the assembly of seed microbiota of field-grown watermelon (*Citrullus lanatus*). We hypothesized that 1) significant overlap exists in the bacterial community composition on flower stigmas and in developing seeds, and 2) bee visitation increases bacterial diversity on flower stigmas and therefore in developing seeds. Using a sequence-based approach (16S rRNA and *gyrB* gene amplicons), we found that 55-87% of seed-associated bacterial taxa were also detected in stigmas. However, community composition differed significantly between stigmas and seeds (PerMANOVA: $F=6.6298$, $df=1$, $p=0.001$), suggesting filtering during floral transmission. Bee visitation of flowers did not significantly affect bacterial alpha diversity on stigmas ($t = -1.6432$, $df = 9.6833$, $p\text{-value} = 0.1324$) or in seeds ($t = 1.3$, $df = 19.897$, $p\text{-value} = 0.2084$). Finally, we tested the impact of bacterial isolates from watermelon stigmas or seeds on seed germination and seedling development under controlled laboratory settings. We found wide-ranging effects on germination and development: for example, normal seedling development was reduced by 59% by a stigma-associated *Rosenbergiella* isolate but increased by 14% by a seed-associated *Bacillus* isolate. Our findings are consistent with a contribution of floral transmission to seed bacterial community assembly in watermelon, with negligible impact of bee visitation. Our findings also show that stigma- and seed-associated bacteria have variable effects on seedling fitness.

seed-associated bacteria, community assembly, floral transmission, insect visitation

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109. Exploring microbial disease dynamics in natural phyllospheres

Paloma Durán, Baptiste Mayjonade, Roxane Lion, Irène Leccia, Rémy Zamar, PATHOCOM Consortium, Fabrice Roux

Despite the rich knowledge on composition of plant-associated microbial communities, little attention has been paid to how microbial communities and environmental variables correlate with microbial disease incidence in natural conditions. Therefore, in the context of the PATHOCOM project, we surveyed *Arabidopsis thaliana* natural populations over multiple seasons and years in the South West of France and observed different levels of disease symptoms across time and locations. Interestingly, environmental variables such as temperature and rainfall correlated with the occurrence of disease symptoms in *A. thaliana* leaves. In order to more closely track microbial fluctuations which may lead to disease in *A. thaliana* plants, we set up a common garden experiment with natural *A. thaliana* accessions, and we collected entire rosettes every week for the whole life cycle of the plant. By sequencing the 16S rRNA marker gene, we observed that while most bacterial groups remained stable over the course of the experiment, the abundance of certain bacterial taxa, including potential pathogenic groups, fluctuated over the life cycle of *A. thaliana*, and this was soil- and accession-dependent. Furthermore, the strongest microbial community changes were observed after a rainfall, highlighting this factor as one of the major drivers of bacterial community composition in plant leaves. To explore this specific hypothesis, we set up another

common garden experiment, and harvested leaf and soil samples before and after a rain event. Microbial community profiles on these samples and the additional assessment of absolute microbial abundances will allow us to investigate the interaction between rain, host genotype and soil composition and their effect on host disease.

plant microbiota, pathobiota, amplicon sequencing, plant disease

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110. Urban particulate matter influences the apple microbiome composition

Matthias Schweitzer, Isabella Kögl, Ahmed Abdelfattah, Wisnu Adi Wicaksono, Gabriele Berg

Urban particulate matter affects health of all organisms on earth, but the impact on the plant microbiome is less understood. Here, we link the Air Quality Index based on particulate matter concentration with the dust and apple epiphytic and endophytic microbiome across the city of Graz. The microbiome of the apple episphere, peel endosphere and pulp endosphere, and additionally dust – was analyzed using amplicon sequencing of the bacterial 16S rRNA and fungal ITS rDNA. Our results show that the fungal communities were more influenced by exposure to air pollution than bacterial communities. We observed significant influence of the air quality on the fungal community structure in the apple episphere and endosphere, and a particulate matter induced selective filtering effect for certain fungal taxa in the dust. On the other hand, bacterial communities were more specific for the individual sample types, especially noticeable in the pulp endosphere. The microbiome of each sample type was comprised of distinct microbial communities dominated by Proteobacteria and Ascomycota, followed by Bacteroidota, Actinobacteriota, and Basidiomycota. Interestingly, a high level of similarity in bacterial community composition was observed between the dust and peel endosphere associated bacterial communities, while the epiphytic bacterial community was significantly different compared to others. Moreover, we suggested that dust could serve as a potential microbial colonization route for the fruit microbiome as most bacteria (55%) colonizing the peel endosphere originated from dust. In conclusion, air quality affects the plant as well as the edible microbiome, which can cause indirect health consequences in humans. Therefore, this knowledge should be integrated in urban and horticultural farming strategies.

Apple microbiome, Urban environment, Air pollution

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111. Temporal and spatial variations in the microbiome of the wild grass *Agrostis capillaris* under different vegetation management practices

Eva Tanneau, Dr. Victor Flores-Núñez, Dr. Janine Haueisen, Dr. Kent Olsen, Prof. Dr. Eva H. Stukenbrock

Microbial communities associated with plants play a crucial role in the health of their host as they can contribute to essential pathways. In natural ecosystems, the plant microbiome is especially known to have a major impact on ecosystem performance and dynamics, as well as improving plant stress resilience. Microbial functions mainly depend on the microbial community diversity and composition, which is itself influenced by numerous environmental

factors. So far, the dynamic of microbiomes in natural plant populations is poorly described beyond studies in the model plant *Arabidopsis thaliana*. Through the study of the grass species *Agrostis capillaris* at Mols Bjerge National Park in Denmark, we aim to study the dynamic of microbiomes and to characterize how vegetation management impacts the microbiome diversity and composition.

Using amplicon sequencing data, we compared the diversity and composition of the plant associated microbial communities in 3 different compartments (leaves, roots and soil) through 3 years of sampling. In addition, we looked at the impact of mowing and grazing on *A. capillaris* microbiome, in comparison to an undisturbed vegetation. Then, we reconstructed the microbial interactions by simulating the community network. Our results highlight a strong impact of the plant tissue on the diversity and microbial composition. Moreover, bacterial communities tend to be stable across 1-year periods, while the diversity and composition of fungal communities change. Finally, even if the management practices seem not to be a main driver of the microbial diversity, differences in abundance and network connectivity were found in certain taxa. We also identified several fungal and bacterial pathogenic species among the most connected individuals in the leaf microbiome communities, including *Zymoseptoria*.

This study represents a first step for investigating the mechanisms shaping the plant microbiome in natural ecosystems, as well as for identifying potential future pathogenic species that could threaten crops.

microbiome, wild ecosystems, grass, microbial ecology, network

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112. Identifying key microbial functions related to maize-microbe interactions using metaproteomics

Anna Garrell, Aisha Mahmood, John Cheadle, John van Schaik, Simina Vintila, Nathan Crook, Ashley Beck, Manuel Kleiner

Plant root-associated microbes have been shown to provide a number of benefits to their hosts, including increased drought tolerance, nutrient availability, and pathogen resistance. While mechanisms behind these beneficial interactions have been and continue to be investigated, there remains a gap in our understanding of the physiology and metabolism of these root-associated microbes in the root environment. In order to address this, we grew members of a seven-species synthetic community from the maize root *in vitro* and *in vivo* and used metaproteomics to identify and quantify genes that are differentially expressed during growth in the root. We identified between 1,507 and 2,159 bacterial proteins from each of the species, with approximately 30-70% of these proteins differentially expressed between the two conditions. Among the proteins found in increased abundance during *in vivo* growth on maize roots, we found many involved in hemicellulose degradation, fatty acid metabolism, chemotaxis and motility, and the Type VI Secretion System. We have investigated several of these functions further in order to understand their role in microbe-root and microbe-microbe interactions using fluorescence imaging, gene knockouts, and *in vitro* assays. Through this work, we have identified proteins that may play important roles in physiological and metabolic functions when grown on maize roots. We additionally found that these functions differed between the seven microbial species, indicating that they may occupy different ecological niches within this environment. These results will have downstream implications for

understanding the mechanisms underlying microbial colonization of plant roots and determining the impacts of abiotic and biotic stresses on plant-microbe interactions.

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113. Plant defense metabolite detoxification by a pathogenic fungus opens necrotic tissue to diverse bacteria in *Arabidopsis thaliana*

Shubhangi sharma¹, Matthew Agler¹

Plants in their natural environments are colonized by a wide range of microorganisms including bacteria, fungi, and other eukaryotes. Among these, bacterial communities in leaves are of particular interest due to their remarkable strain-level diversity, which holds significant implications for plants. A diverse bacterial community is more resilient and better equipped to handle environmental stresses and disease outbreaks. It is known that both plant host factors and microbe-microbe interactions, in particular involving plant pathogens, play key roles in filtering bacterial communities in leaves. Aliphatic glucosinolates (GLSs) are plant metabolites synthesized by Brassicaceae plants, which play a crucial role in defense against herbivores. GLSs are activated by myrosinase-mediated hydrolysis of glucosinolates upon tissue damage, resulting in the formation of defence metabolites, such as isothiocyanates (ITCs). Additionally, the necrotrophic leaf-colonizing fungal pathogen *Sclerotinia sclerotiorum* (SSc) can also induce significant changes in leaf physiology and chemical composition by releasing ITCs during necrosis and hydrolysing them. In this study, we investigated the role of glucosinolates in controlling how SSc influences the bacterial communities in leaves. Experiments were conducted with *Arabidopsis thaliana* in the Col-0 Wt background and its myb28/myb29 mutant, which lacks the ability to synthesize glucosinolates. Subsequently, the plants were infected with the necrotrophic fungus *S. sclerotiorum* or its Δ SScSaxA mutant, which cannot hydrolyse ITCs. 16S rRNA gene amplicon sequencing revealed significant fungal-mediated changes in the commensal leaf microbiome, which was influenced by the presence of glucosinolates and the fungus's ability to hydrolyse ITCs. These results highlight that the rearrangement of leaf bacteriomes by fungal pathogens can depend strongly on leaf secondary metabolite chemical defences and their transformations by the fungus. Further investigations will determine the implications of these dynamics for fungal pathogenicity.

Glucosinolates, Fungal pathogen, Plant defence, Bacterial community

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114. Beneath the agroforest floor: understanding the role of litter-associated fungi community structures and functions under agroforestry systems in the Eastern Amazon

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One of the main drivers of soil microbial activity is the soil organic matter and its chemical properties, which are highly dependent on aboveground tree identity and diversity and their carbon and nitrogen inputs. This is especially important for agroforest systems (AFS) in the

Amazon, which rely on a complex network of plant-microbe interactions for effective nutrient cycle from the litter. Here, we study the role of AFS age on the litter-associated fungi community composition and functionality. We hypothesize that with increasing AFS age can restore the fungi communities and functionality to levels closer to the ones found in native forests. For this, we sampled the litter layers from five sites, ranging from a pasture to 7, 35, and 55-year-old AFS to an undisturbed primary forest in the eastern Amazon. We quantified the role of AFS age in the litter inorganic chemical profile, litter carbon and nitrogen content, and litter fungi community composition and their functional guilds. Our findings revealed the influence of AFS age has a significant impact on fungal community alpha and beta diversity, increasing the presence of symbiotrophs with AFS age in 24.9%, while saprotrophs and pathotrophs were suppressed by 10.1% and 30.3%, respectively. Along the time gradient, AFS age changed the litter nutrient content profile and was positively correlated with the changes in fungal guild composition ($R^2 = 0.60$; $p\text{-value} < 0.001$). Furthermore, AFS diversity had a strong positive correlation with fungal guild distribution, as younger AFS were less associated with complex substrate degradation, while older AFS presented a stronger association with wood and leaf degradation. These findings highlight the contribution of AFS in restoring the above-belowground interactions, and the role of AFS age in litter-associated fungi community composition, and its effects on their guilds.

Tropical agroforestry; Tree diversity; Litter fungi community; Molecular ecology; Fungal guilds
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115. Tar Spot Disease Severity Influences Phyllosphere-Associated Bacterial and Fungal Microbiomes

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Tar spot, caused by the obligate fungal pathogen *Phyllachora maydis*, is a foliar disease of corn that has become a major economic concern in the United States. To test the hypothesis that *P. maydis* can interact with other foliar microorganisms, we investigated phyllosphere microbiomes in relation to corn inbreds with differential tar spot symptoms under natural infestation in the field. Leaf samples from sixteen inbred lines were assessed for tar spot symptoms, and bacterial and fungal microbiomes were characterized by paired-end sequencing on the Illumina MiSeq platform. Comparison of the phyllosphere microbiomes revealed distinct bacterial and fungal communities between resistant and susceptible lines. Bacterial and fungal species richness was significantly higher in resistant compared to susceptible inbred lines in a sample-specific manner. In contrast, there were no clear differences in diversity when including evenness of bacterial communities between the resistant and susceptible lines. Diversity of fungal communities differed significantly, particularly between twelve of the fourteen susceptible lines versus resistant lines. Plant-beneficial bacterial genera such as *Methylobacterium* and *Quadrifidaria* were associated with resistant lines, while *Pantoea*, *Deinococcus* and *Pseudomonas* were the least abundant. The second-most commonly detected fungus likely was a *Coniothyrium*, but whether it is the same species that was identified as a possible mycoparasite of *P. maydis* in Central and South America is not known. Fungal genera *Cladosporium*, *Papiliotrema*, *Cryptococcus*, *Tilletiopsis* and *Alternaria* were associated with resistant lines while *Sphaerellopsis* was the least-abundant genus. In contrast, *Puccinia*, *Sphaerellopsis* and *Phyllachora* were the dominant fungal genera in susceptible lines. Our findings imply that *P. maydis* infection may result in a distinct microbiota with lower diversity. Further analyses of these distinct microbiota between

resistant and susceptible lines could lead to a better understanding of the potential role of foliar microbiomes in causing or resisting *P. maydis* infection.

maize, microbial diversity, microbiome, phyllosphere, tar spot, *Phyllachora maydis*

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

116. Unearthing the Secrets of the Rhizosphere: Unlocking the Potential of Microbial Functional Genes for Drought-Resistant Agriculture

Ana Vitória R. da Silva, Izadora C. M. Cunha, Thierry A. Pellegrinetti, Eduardo M. Boleta, Teresa M. L. Mafra, Francisco D. Andreote, Rodrigo Mendes, Siu M. Tsai, Lucas W. Mendes.

Water stress poses a significant challenge to global agricultural productivity, impacting major agricultural regions worldwide through drought conditions and adversely affecting economies and societies. Recognizing the importance of enhancing crop resilience to drought, recent attention has been directed towards genetic improvements for drought tolerance. Emerging research highlights the role of soil microorganisms in influencing the plant's ability to withstand drought stress. This study aimed to investigate the rhizosphere microbiome of the common bean, aiming to identify microbial functions that contribute to the plant's resilience to water stress. To achieve this goal, we cultivated both drought-tolerant (BAT-477 and SEA-5) and susceptible (IAC Milenio and IAC-Carioca 80-SH) common bean cultivars in a greenhouse experiment under drought conditions for 96 hours. Measures including plant height, biomass, gas exchange, and nutritional content, alongside molecular analysis of rhizosphere soil through metagenomics sequencing, were used to evaluate the functional profile of the microbiome. Our initial findings reveal that tolerant cultivars showed an increase in microbial functions associated with biofilm formation and long-term survival in the dormant state. Conversely, susceptible cultivars displayed an enhanced abundance of membrane-associated proteins, signaling, and cellular processes, including DNA replication/repair and microbial metabolism in extreme environments under drought stress. Notably, co-occurrence network analysis revealed that, under stress conditions, the tolerant (SEA-5) and susceptible (80-SH) cultivars experienced increased modularity and a greater number of communities linked to the functional profile. Species diversity was particularly marked in one tolerant (BAT-477) and one susceptible cultivar (80-SH), pointing to complex interactions between plant types and their rhizosphere microbiomes. These insights significantly advance our understanding of plant-microbe interactions, highlighting microbial functions that could be harnessed for biotechnological solutions to enhance drought resilience. Moreover, this research underscores the critical need for further microbial ecology studies to innovate sustainable agricultural practices.

Rhizosphere, microbiome, drought, functional genes, metagenomics.

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117. Synergistic effects of synthetic microbial communities on soybean drought tolerance

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Drought poses a significant abiotic stress threat to crop production, with certain root-associated individual bacteria demonstrated to mitigate its negative effects on plant growth. However, the effects of synthetic microbial community on drought tolerance of Soybeans and the underlying mechanisms remain largely unexplored. Here, we compared the variation in community composition of rhizosphere and endophytic bacteria between drought-sensitive and -resistant soybean varieties to identify the potential keystone phylotypes capable of enhancing plant adaptability to drought stress. A synthetic community (SynCom) with eight keystone species was constructed to examine their effects on the physiological responses of sensitive varieties to drought. We observed that SynCom remarkably improved Soybean biomass under drought conditions compared to the no-drought treatments (with/without SynCom), indicating the beneficial effect of SynCom is specific to drought conditions. In addition, no effect was observed when subjected to the individual strains, despite their ability to colonize the root surface (confirmed through field emission scanning electron microscopy and fluorescence labeling techniques), suggesting that the emergent property of SynCom was the underlying cause of induced drought tolerance. Plant-associated transcriptome and metabolome analyses showed that the enhanced drought tolerance induced by SynCom inoculation was related to the destruction of root ion balance homeostasis and mediation of histidine and alanine metabolism. SynCom-associated metatranscriptomic and metabolomic results indicated that SynCom under in vitro drought conditions mainly enriched functions related to heterocyclic binding, catalytic activity, and the production of N-Acetylcarnosine. In addition, SynCom can synergistically produce more Indole acetic acid and recruit more iron ions under drought conditions. Our findings may provide valuable insights for future endeavors aimed at engineering emergent bacterial community properties to enhance plant tolerance to drought.

Plant microbiome; SynCom; Drought; Soybean; Emergent property

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118. The role of auxiliary plants microbiome in assisting *Triticum aestivum* to withstand extreme climatic conditions.

Mathieu Vanhove, Cendrine Mony, Philippe Bandenkoornhuysen

"Current climatic predictions and the human population increase represent tremendous challenges for global agro-ecosystems which need to double their production by 2050 while facing extreme climatic events such a drought, heat, or the spread of pathogen. Therefore, agriculture needs to use ecological services which exact functions and dynamics remain to be fully unveiled. Investigating plant-microbe interactions, specifically the benefits of microorganisms and their involvement in crop production, are becoming more and more crucial to mitigate these deleterious effects. In agro-ecosystem, considering the impact of neighboring plants onto the cultivated plant appear as a paradigm shift. Microbes are involved in a multitude of ecological functions ranging from soil fertility, tolerance to environmental stresses

or diseases resistance. In this context, endospheric microbial communities associated with plants can vary due to environment factors as well as plant phylogeny or due to the presence of neighboring plants. We investigated the role of various microbiomes associated with a wide range of plants species (n=29) onto the wheat (*Triticum aestivum*) microbiome and wheat phenotype. The structure of microbial assemblages associated with a focal plant depends on the identity of the neighboring plant species. Next, we characterized the role of microbial roots transfer from auxiliary plants onto our focal plant in order to withstand drought. We tested a subset of plants which were found to impact the wheat phenotype and provided fungal and bacteria enabling drought-stress tolerance. This work was performed under controlled conditions (green house). Plant phenotype was examined and the microbial assemblage characterized and compared with plant traits."

Neighboring plants; microbial assemblage; microbiome transfert

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119. Integrating microbiota towards a robust stimulation of plant growth and health with PGPR

Martin QUIÉVREUX, Marc ONGENA, Philippe JACQUES, Caroline DE CLERCK, Sébastien MASSART, Pierre DELAPLACE

"Soil and plant health are inextricably connected to agriculture, and root systems play a major role in the growth and health of cultivated plants. They are in constant interaction with microorganisms and are at the core of water and mineral nutrition. Also, agriculture needs to decrease the use of synthetic pesticides and fertilizers. In this context, biostimulation and biocontrol agents could help pave the way towards a more sustainable agriculture. However, their efficacy varies depending on environmental conditions, and soil microbial communities represent a factor of variability.

This context raises questions about the interactions between plant development, soil microbiota, and beneficial microorganisms. For this, the RootBoost project aims at addressing two issues related to the efficiency of Plant Growth-Promoting Rhizobacteria (PGPR) in agriculture:

The project aims to understand how PGPR influence plant development and their interactions with soil microbiota. Experiments will assess the impact of *Bacillus* sp. strains on tomato and wheat growth, health, and root development, as well as associated microbial communities in various substrates. Multi-omics techniques will be employed to examine how PGPR efficacy is modulated by environmental conditions, such as soil microbial diversity. The developed experimental models and preliminary results will be presented in the aforementioned context.

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PGPR, soil microbiota, rhizosphere, plant growth

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120. Undermining the “cry for help”: how a phytopathogenic fungus undermines host recruitment of antagonistic bacteria

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Plant pathogens secrete small cysteine-rich proteins called effectors during infection to promote disease development through various mechanisms. While effectors were typically thought to target host physiology, including host immune responses, we discovered that some effectors target the microbes that live on and in the plant to promote host colonization. More specifically, the soil-borne fungal plant pathogen *Verticillium dahliae* secretes several effectors with selective antimicrobial activity that target microbial antagonists in the microbiota of its plant hosts. We now present the functional characterization of a novel effector that displays antimicrobial activity, named Av2. Deletion of Av2 compromises the virulence of *V. dahliae* in planta, as tomato plants inoculated with the wild-type strain developed stronger symptoms of disease than plants inoculated with the deletion strain. Intriguingly, inoculation experiments using a gnotobiotic plant cultivation system revealed a microbiota-dependent virulence contribution of Av2. To investigate the effect of Av2 during host colonisation we performed amplicon sequencing and showed that Av2 modulates the host microbiota by specifically suppressing the recruitment of Pseudomonadales. Co-cultivation experiments revealed that Av2 deletion leads to reduced *V. dahliae* growth in the presence of particular plant-associated *Pseudomonas* spp. Furthermore, the same antagonistic *Pseudomonas* spp. bacteria display sensitivity to Av2 in vitro. Altogether, we propose that *V. dahliae* secretes Av2 to manipulate the microbiota of its host plants by undermining the “cry for help” recruitment of beneficial *Pseudomonas* spp. to ultimately promote disease development.

Plant-pathogen interaction, antimicrobial protein, microbiota manipulation, fungal-bacterial interaction, intermicrobial interaction, antagonism

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121. Bacteria-Mediated Salt Stress Alleviation in Rice

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Rice (*Oryza sativa* L.) is one of the most important staple foods in the world and a major source of income for millions of people. However, rice cultivation is heavily affected by climate change. Drought and seawater intrusion has led to salinization of rice paddies resulting in significant yield losses across Europe and Asia. Root-associated microbes have been reported to enhance protection against abiotic stresses such as salt stress. We established a large collection of root-associated bacteria from a hypersaline rice paddy located in the Guadalquivir area near Isla Mayor, Spain. We developed and optimized a high-throughput screening for bacteria-mediated salt stress alleviation in rice. Using this high throughput screening we aim to identify novel bacteria which can alleviate salt stress in rice seedlings and increase germination and growth. Preliminary results demonstrate the efficacy of *Cellulomonas* and *Alkalihalobacillus* isolates in alleviating salt stress in rice seedlings in vivo. This research aims to elucidate the underlying mechanisms involved in salt stress alleviation by bacteria. Understanding these mechanisms will provide valuable insights into the interactions between root-associated bacteria and rice plants under saline conditions, offering new paths for rice cultivation in salt-affected regions.

Salt stress, Rice, abiotic stress

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122. Utilising the Seed Microbiome of *Beta vulgaris* as a Biological Resource for Beneficial Bacteria

Joseph King, Jake Chandler, Tony Pawlik, Nora Temme, Gerhard Leubner-Metzger

The seed microbiome is the combination of its surface (epiphytes) and tissue (endophytes) associated bacteria or fungi. Forming close-knit relationships to plants post-germination, the seed microbiome directly influences community composition of mature plants in the rhizosphere, phyllosphere and endosphere. Being subject to vertical inheritance from maternal plant, the seed microbiome is of evolutionary importance equipping progeny with communities for optimal growth, abiotic stress resilience and resistance to disease. The seed has therefore been proposed as a biological resource for beneficial bacteria and fungi, but molecular functioning of the seed microbiome in the field is generally unknown beyond a sequencing-based approach elucidating composition. Having developed a protocol to isolate specific enzyme expressing bacteria from the sugar beet (*Beta vulgaris*) seed microbiome, this project aims to address the role of native beneficial bacteria during early seedling development and improve growth under osmotic stress using a biopriming approach. Understanding and utilising the seed microbiome will be an indispensable tool for developing sustainable agricultural practices improving tolerance to abiotic and biotic stressors in a rapidly changing environment due to climate change. Furthermore, obtaining isolates from seed may provide more appropriate treatments increasing abundance of already well-established taxa.

Seed Microbiome, Beneficial Bacteria, *Beta vulgaris*, Biopriming

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123. Microbial Induction of Plant Resilience to Drought Stress (MicroRes)

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In the past decades, yields of major food crops worldwide have decreased due to drought. Over the past years, it has become evident that microorganisms associated with plants can enhance drought tolerance in various ways, allowing better crop growth under water-limiting conditions. It has been shown that by producing secondary metabolites like EPS, osmolytes and hormone precursors, bacteria enhance plant growth under drought stress. In this study we isolated a total of 146 bacterial strains from a salty and nutrient deficient soil of the island of Terschelling (The Netherlands) and tested them for their ability to alleviate drought stress in *Arabidopsis thaliana*. Among the collection, 12 bacteria belonging to the phyla Bacteroidota (4), Actinobacteria (5) and Proteobacteria (3) exhibited drought stress tolerance enhancement. The isolate N122 identified as *Pseudomonas* sp. showed an in planta drought-stress alleviation effect through volatile organic compounds (VOCs). Microscopy revealed that plants exposed to the VOCs of N122 significantly enhanced root hair formation. Currently we are analyzing the N122 volatile blend using gas chromatography (GC-MS) and screening a $tn5$ mutant library to identify the volatile compounds responsible for the drought alleviating effect together with the

underlying genetic background. The beneficial effects of bacterial volatiles offer an innovative way of alleviating drought in crops that opens new perspectives for sustainable agriculture in the age of climate change.

Drought stress,

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124. Identifying Arabidopsis candidate genes involved in its response to plant-growth promoting rhizobacteria

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"Pseudomonas simiae WCS417, a Plant-growth promoting rhizobacteria (PGPR), enhances Arabidopsis thaliana growth by colonizing roots, leading to morphological changes in both roots and shoots. These changes vary among Arabidopsis accessions, indicating natural variation in responsiveness to WCS417. The aim of this research is to identify candidate genes involved in the interaction between Arabidopsis and WCS417. A Genome-Wide Association Study (GWAS) will be conducted on a large subset of Arabidopsis accessions from the 1001 Genome Project. The large amount of phenotypic data required for the GWAS will be generated using the image-based, high-throughput phenotyping technology from the NPEC facility at Utrecht University.

The current aim in this research is to find interesting phenotypic traits that can be observed using the NPEC facility. An in vitro root phenotyping system pipeline has been set up that can show root colonization of a fluorescent mCherry WCS417 strain. Additionally, through imaging Arabidopsis plants in vivo, a plant-growth promoting effect of Arabidopsis shoots has been shown through quantifying plant area. The root colonization and quantification of plant area could serve as potential phenotypes for the GWAS that will be performed in the future. "

WCS417, PGPR, GWAS, phenotyping, Arabidopsis

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125. The Role of Bacillus in Modulating Nitrogen Effects on Arabidopsis Growth

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Nitrogen stands as a pivotal nutrient crucial for plant growth. Plants commonly draw upon ammonium and nitrate as primary inorganic nitrogen sources. An imbalance in the supply of these forms in soil or growing media often leads to suboptimal plant growth. While Bacillus species have been used as growth-promoting bacteria, helping plants resilience against diverse stresses such as salinity and pathogen attacks, their role in mediating the impact of nitrogen balance on plant growth remains understudied. To study this, we isolated several Bacillus strains and characterized their function in modulating the effects of nitrogen on Arabidopsis thaliana growth in MS medium. Initially, we established three nitrogen concentration gradients: 0 mM, 1 mM, and 5 mM using NH₄NO₃ as the nitrogen source. Bacillus strain JA18 displayed significant growth-promoting effects across all nitrogen gradients, compared with control

without *Bacillus* inoculation. Subsequently, to assess *Bacillus*' impact on plant growth under varied nitrogen source supplies, five different ammonia/ nitrate nitrogen concentration ratios were used (0:100, 25:75, 50:50, 75:25, and 100:0). Interestingly, *Bacillus* strain growth-promoting effects were only evident in the presence of ammonia-nitrogen, with no enhancement observed under sole nitrate-nitrogen supply condition. Particularly, under conditions of 100% ammonia, *Arabidopsis* growth is inhibited compared to the other four conditions. However, inoculation with *Bacillus* counteracts this growth inhibition. These findings highlight a novel role of *Bacillus* in mediating the impact of nitrogen balance on plant growth, suggesting potential avenues for future research and application in agricultural settings.

Bacillus, *Arabidopsis thaliana*, nitrogen, growth promoting, stress tolerance.

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126. Using seed microbiota to improve seedling phenotype

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The microbial communities associated with seeds, known as seed microbiota, are involved in seed germination, seedling emergence, and seedling health. Understanding the assembly, composition, and function of seed microbiota is therefore essential for optimizing agricultural practices and increasing crop productivity. Recent advances in seed microbiota engineering allow the manipulation of seed microbiota to better understand the role of selected strains and representative microbiota on seed germination, seedling emergence and seedling phenotype.

The seed microbiota is a low-diversity community with dominant strains found across multiple species and agricultural conditions. Here, we evidence the role of bean seed natural microbiota in seed germination and seedling emergence ability across eight genotypes grown in field conditions and identify bacterial (Microbacteriaceae, Rhizobiales, Pseudomonadaceae) and fungal taxa (Leotiomyces, Dothideomycetes, Sordariomycetes) associated with seed emergence potential. Then, using synthetic communities inoculations, we confirm the influence of seed-borne microorganisms and soil-dependent effect of seed inoculation on seedling phenotypes. We show associations between seed or seedling microbiota and specific plant metabolites at different early developmental stages likely involved in plant-microbe interactions. Native seed strains from the Microbacteriaceae, Enterobacteriaceae, Pseudomonadaceae, Xanthomonadaceae, Bacillaceae, Erwiniaceae, Sphingomonadaceae, Sphingobacteriaceae, Oxalobacteraceae, Weeksellaceae and Rhizobiaceae families were inoculated in consortia. More than 200 metabolites were identified in plant samples.

Ultimately, this approach could be used to design and inoculate synthetic microbiota based on desired plant phenotype traits, such as resistance against phytopathogens.

Seed microbiota, synthetic communities, plant metabolomics

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127. Growth substrate influences seed endophytic bacteria community and seed germination in three successive Arabidopsis generations

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The seed microbiome is recognized as indispensable for the initial phases of plant development, notably seed germination, with soil serving as the primary source for shaping seed microbial communities. In this study, Arabidopsis accessions were cultivated across three successive generations in a controlled greenhouse environment, employing four different growth substrates. Progeny seeds, subjected to surface sterilization, were utilized for germination assays under salt and osmotic stress conditions, alongside the analysis of seed endophytic bacterial populations via targeted 16S rRNA gene sequencing (V5-V7 region), employing a PNA Clamp to mitigate host plant DNA amplification. Our findings reveal a significant enhancement in germination percentage under salt and osmotic stress for seeds derived from plants grown in field soil (MiCROP soil) after three generations of growth, compared to those from plants cultivated in gamma-sterilized soil. Conversely, a discernible decrease in bacterial diversity, particularly the absence of the Firmicutes phylum, was observed in third-generation seeds (G3) from plants cultivated in field soil (MiCROP soil) in contrast to those from gamma-sterilized soil. Likewise, some bacterial taxa are depleted in third-generation seeds compared to the parent seeds (G0). These observations underscore the significant influence of host plant growth substrate on seed microbial diversity. However, this observation challenges the conventional hypothesis, suggesting that the association between increased microbial diversity and enhanced plant performance may not hold true for the seed microbiome, particularly in scenarios where optimal microbial selection is imperative amid resource constraints. We aim at isolating seed endophytic bacteria from third-generation seeds, and investigating their potential to stimulate Arabidopsis seed germination and seedling growth under abiotic stress.

seed microbiome, seed germination, bacterial seed endophyte, salinity, osmotic, growth substrate

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128. Plant coumarin-fungal endophyte interactions and the impact on Arabidopsis iron nutrition

Lara Van Dijck, Jane E. Parker

Plants shape their root microbiome with the help of various root-exuded compounds (Stassen et al., 2021). One class of such molecules are the coumarins. These secondary metabolites, derived from the phenylpropanoid pathway, are ubiquitous across plant species and are also present in marine organisms, bacteria, insects and fungi (Stringlis et al., 2019). Several coumarins, such as scopoletin, exhibit selective antimicrobial properties, suggesting their role in microbial selection within the endo- and rhizosphere (Robe et al., 2021). Coumarins also play a role in the mobilization of iron from the soil (Paffrath et al., 2023). The exudation profiles of coumarins and their variable activities in reducing or mobilising iron is influenced by multiple factors such as pH and iron availability, and the presence of microbes (Pieterse & Stringlis, 2023). Harbort et al (2020) demonstrated that root commensal bacteria are able to rescue iron-starved Arabidopsis plants in a process that is dependent on secretion of plant-derived

coumarins. Building on these findings, our research aims to explore whether commensal fungal root endophytes can improve iron nutrition in Arabidopsis plants under varying iron availabilities. Also, we are examining the impact of coumarins on fungal growth and root colonisation. By focusing on the interaction between endophytic fungi and coumarins, this project aims to unpick mechanisms in the host and fungal endophyte which enhance plant iron nutrition, and thus nutritional resilience. Our objective is to deepen understanding of the intricate interplay between coumarins, the microbiota and plant roots in the fight for iron.

root-exudates, coumarins, iron, fungal endophytes

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129. Arabidopsis growth, defense and root microbiota are modulated on soil conditioned by maize root exudates

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Maize plants exude secondary metabolites known as benzoxazinoids (BXs) through their roots, which can alter the soil and root microbiota and the performance of subsequently grown maize or wheat plants. To further investigate the underlying mechanisms of such BX-driven plant soil feedbacks, we studied the response of the model plant *Arabidopsis thaliana* (henceforth *Arabidopsis*) to BX-conditioned soil. We found that the *Arabidopsis* accession Col-0 grew bigger on soil conditioned with BX-exuding maize compared to the control soil conditioned with bx1 mutant maize defective in BX biosynthesis. The bacterial community composition of *Arabidopsis* roots was differentiated when grown in control soil compared to BX-conditioned soil, and growth on sterilized soil revealed that the soil microbiota is involved in shaping the growth feedback. In shoots grown on BX-conditioned soil, we analyzed the transcriptome and found upregulated clusters of genes related to induced resistance responses mediated via the hormones jasmonic acid and salicylic acid. When we infected *Arabidopsis* leaves with the necrotrophic fungus *Botrytis cinerea*, smaller lesions developed when plants were grown on BX-conditioned soil. With our ongoing work we further investigate whether this increased defense is based on defense priming. Furthermore, in the root transcriptome we found members of the GRF transcription factor family, which are known to be involved in the plant growth/defense trade-off, to be upregulated on BX-conditioned soil.

We found that *grf1* and *grf3* mutants did not display a BX-conditioning dependent growth response. Furthermore, *grf1* mutants grown on BX-conditioned soil harbor a decreased expression of genes involved in response to salicylic acid in the roots compared to roots of Col-0 plants grown on BX-conditioned soil. Together, these findings imply that soil conditioning by maize BXs modulates the root microbiota and alters *Arabidopsis* growth and defense, and that GRF members might be involved in this regulation.

Plant-soil feedbacks, benzoxazinoids, *Arabidopsis thaliana*, root microbiota, growth-regulating factors

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130. Initial Soil pH and its Impact on the Dynamic Adaptation of Rhizosphere Microbiomes to Mineral Nitrogen Forms

Maywald, N. J.; Hernández Pridybailo, A.; Francioli, D.; Ludewig, U.

Soil microbiomes, which are essential for maintaining agricultural ecosystems and supporting crop vigour, have a significant influence on plant health and productivity. However, the complex responses of soil microbiomes to different forms of nitrogen, such as ammonium or nitrate, are influenced by a variety of factors, including initial soil pH and site-specific conditions. This variability makes it difficult to generalise the effect of nitrogen form on complex plant-microbe interactions and to draw precise conclusions about the specific effects of a particular nitrogen form on interactions between plants and potentially beneficial or pathogenic microbes. In this study, we investigated how initial soil pH affects the ability of ammonium and nitrate to influence microbial communities within the rhizosphere of maize plants. Soils were collected from two different field sites. One site was of particular interest due to the presence of a natural pH gradient ranging from 5.98 to 7.45, while other soil characteristics remained relatively constant. The soil from the second site had a similarly low pH of 5.89, which additionally was artificially adjusted to 7.39 using potassium hydroxide. We found that while initial soil pH remains the primary factor influencing microbial populations, the form of nitrogen has a remarkable ability to reshape bacterial and fungal communities within the rhizosphere in as little as three weeks. The nitrogen source affected the composition of the bacterial community at both low and high initial soil pH, while the composition of the fungal community was particularly affected at low pH. To gain a thorough understanding of these shifts and their implications for crop production, we conducted in-depth analyses of plant performance, subsequent pH changes, root exudates and the plant transcriptome. This approach aims to improve the current understanding of plant and rhizosphere microbiome adaptations to nitrogen dynamics.

Ammonium, nitrate, plant nutrition, beneficials, pathogens, plant performance

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131. A case of conditional pathogenesis: An unusual interaction between *Arabidopsis thaliana* and a beneficial rhizobacterium *Pseudomonas putida* AKMP7 under water stress.

Raja Gopalan N.S., Raunak Sharma, Yegna Priya and Sridev Mohapatra.

Plant growth-promoting rhizobacteria (PGPR) are beneficial soil bacteria that reside in the rhizosphere and can colonize the roots of plants in their vicinity. Although, studies on the beneficial impact of PGPR have a long history, research on the behaviour of beneficial microbes that turn harmful to their host due to changes in environmental conditions remain non-existent. Research from our laboratory has uncovered a unique phenomenon that we have termed “conditional pathogenesis” in which, a particular strain of drought and thermo-tolerant PGPR, namely, *Pseudomonas putida* AKMP7, becomes pathogenic towards *Arabidopsis thaliana* under drought conditions, and causes early wilting of plants. Contrastingly, it can promote plant growth under well-watered conditions in the model plant, *A. thaliana*, and, under both conditions, in the model crop, *Oryza Sativa*. In this study, with an extensive analysis of bacterial-secreted phytohormones, we unveiled that AKMP7 releases (a) free zeatin, (b) auxin derivatives such as indole-acetamide and (c) amino acid (alanine, phenylalanine, and

aspartate) conjugates of auxin into the growth medium. Interestingly, the levels of most of these phytohormones were higher under osmotic/water-stressed conditions than under non-stressed conditions. Through studies involving the treatment of plants with the specific phytohormones/derivatives/conjugates mentioned above, we have identified that zeatin and the auxin derivative, indole-acetamide can play a crucial role in aiding the drought-specific adverse effects of AKMP7 in *A. thaliana*. Apart from this, we have also studied the regulation of plant immune response during conditional parthenogenesis and identified that AKMP7 negatively regulates the salicylic acid-mediated plant immune response to cause early wilting of plants under water stress. Overall, our work lays a foundation for mechanistic understanding of the unique phenomenon of conditional pathogenesis.

Pseudomonas putida AKMP7, *Arabidopsis thaliana*, conditional pathogenesis, water stress, bacterial phytohormones, plant immunity

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132. Exploring *Pseudomonas* communities in disease suppressive composts using genus-specific long-read rpoD amplicon sequencing

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The application of compost is considered as an environmentally friendly means to suppress soilborne diseases, but its potential is still far from being fully utilised, because of the high variability among different composts and the unpredictability of its efficacy. We aim to understand the factors influencing the disease-suppressive activity of compost and analysed over forty composts for their disease suppression in different plant-pathogen systems, their physico-chemical properties, and their microbial communities. The compost microorganisms are key to the disease suppressive activity, and through 16S amplicon sequencing we identified several bacterial genera that are indicative of highly suppressive composts. However, known biocontrol genera, such as *Pseudomonas*, can be very versatile, harbouring phylogenetically closely related strains with contrasting characteristics. Short-read sequencing of the 16S gene does not provide sufficient resolution of such a genus, e.g., we found *Pseudomonas* in composts of all suppression levels, but were unable to describe them to the species level. Therefore, we employed a recently developed rpoD-based PacBio long-read amplicon sequencing method, which allows for sub-species resolution of *Pseudomonas* communities, to investigate the *Pseudomonas* communities in the rhizosphere of cress and cucumber plants grown with different composts. Our analysis revealed that *Pseudomonas* communities of cress grown in disease-suppressive composts differed significantly from those of cress grown in non-suppressive composts. We found two ASVs indicative of disease suppression that were not detected by the 16S-based short-read approach, highlighting the value of long-read sequencing for identifying potential biocontrol strains. Rhizosphere *Pseudomonas* communities did not differ significantly between healthy and diseased plants, but we found indications for differences between the two host plant species. Our results show that high-resolution taxon-specific amplicon sequencing is a very valuable addition to general microbial community analysis, which in this study allowed to identify members of *Pseudomonas* communities, which are indicative for disease suppressive composts.

PacBio long-read sequencing, Biocontrol, Soilborne diseases, Plant-beneficial *Pseudomonas*, *Globisporangium ultimum*

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133. Environmental pH shapes coumarin-dependent interactions between the root microbiota and *Arabidopsis thaliana* to maximise iron nutrition

Milena Malisic, Charles Copeland, Anton Amrhein, Pengfan Zhang, Sabine Ambrosius, Stanislav Kopriva, Ruben Garrido-Oter, Nicolaus von Wirén, Ricardo F.H. Giehl, Paul Schulze-Lefert

The roots of *Arabidopsis thaliana* secrete secondary metabolites called coumarins that cooperate with the bacterial root microbiota in mobilising iron in alkaline soils (Harbort et al., 2020). The mobilised iron is then taken up into the root via a dedicated reductive import machinery, consisting of the ferric iron reductase FRO2 and the ferrous iron importer IRT1. In acidic soils, the contribution of the root microbiota to plant iron nutrition and its interaction with the reductive import pathway remains uncharacterised. Here we examined the respective contribution of and interactions between (1) plant-derived coumarins, (2) reductive import of iron, and (3) the root microbiota under acidic pH. We found that the majority of taxonomically diverse bacterial members of the *Arabidopsis* root microbiota alleviate host iron deficiency in a coumarin-dependent manner. However, depending on the environmental pH, distinct coumarin structure types and functions engage in cooperative interactions with the root-associated bacteria resulting in either increased ferric iron mobilisation or ferric iron reduction. The latter eliminates the need for FRO2-mediated iron reduction, which is essential to *Arabidopsis* iron acquisition in a germ-free context. Collectively, our work suggests that in alkaline and acidic soils, *Arabidopsis* preferentially utilizes different coumarin structure types and the bacterial root microbiota to maximise iron acquisition. More generally, we contend that the bacterial root microbiota should be considered an integral component of *Arabidopsis* iron nutrition.

Root microbiota, *Arabidopsis*, plant iron nutrition, coumarinseither

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134. Legume rhizodeposition promotes nitrogen fixation by soil microbiota under crop diversification

Mengjie Qiao, Michael Bonkowski, Yan Chen

While biological nitrogen fixation plays a key role in sustainable crop production, how this process is influenced by the interactions between legumes and coexisting crops is poorly understood. Here, we elucidated how these interspecific effects are shaped in the legume rhizosphere, via metabolite deposition and functional responses of free-living and symbiotic nitrogen-fixing bacteria. Based on a long-term diversified cropping field experiment, we found that peanut co-cultured with maize and oilseed rape led to specific changes in peanut rhizosphere metabolite profiles. Flavonoids and coumarins accumulated due to the activation of phenylpropanoid biosynthesis pathways in peanuts, which enhanced the growth and nitrogen fixation activity of free-living bacterial isolates and root nodulation by symbiotic *Bradyrhizobium* isolates that were typical of the peanut rhizosphere in the most diverse (peanut/maize/rape) cropping system, thus improve peanut biological nitrogen fixation. Our

findings demonstrate that a smart selection of crop species can be applied for the targeted manipulation of rhizosphere microbiomes and their functionality to improve nitrogen availability in sustainable agricultural systems.

Plant recognition, Soil chemical legacy, Nutrient accumulation

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135. A plant's diet is essential for plant communication in the rhizosphere and symbiosis establishment

Lopez-Raez JA, Lidoy J, España L, Marro N, Rial C, Ramos A, Macias FA, Pozo MJ

In the current scenario of global climate change, one of the major challenges for modern agriculture is to increase food production while protecting the environment. Crops are continually exposed to different types of stresses, which significantly impact their productivity. Among them, the plant's nutritional status is one of the most critical limiting factors. Phosphorus (P) and nitrogen (N) are essential macronutrients that are integral to plant growth and development, being widely used as chemical fertilizers. Plants optimize P and N uptake through a series of physiological adaptations and through their engagement with beneficial microorganisms present in the rhizosphere, such as arbuscular mycorrhizal (AM) fungi and rhizobacteria. The use of these plant's partners as biostimulants is gaining increasing interest in the agri-food sector due to their potential as biofertilizers and bioprotective agents. However, despite their potential, their implementation in agricultural settings is still challenging because of the variability of results in the production systems. Plant-microbe interaction requires a very precise communication, which is based on the production and exudation into the rhizosphere of secondary metabolites that act as cry for help cues. Among these molecular signals, strigolactones and flavonoids play a paramount role. In the present work, we will address the key role of the plant's P and N status in the regulation of the production and exudation of these molecular cues, and their relevance for the proper establishment of AM symbiosis. In addition, the cross-talk between these two important plant biosynthetic pathways will be discussed. This knowledge will help to implement the use of beneficial microorganisms, including AM fungi, as biostimulants and to optimize the use of agrochemicals in modern and sustainable agriculture.

Plant communication, Beneficial microorganisms, Rhizosphere signaling, Nutritional status, Strigolactones, Flavonoids

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136. Effect of green cover crops on the soil microbial biodiversity of an olive trees field in Spain

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In this work, the international LIVINGRO® protocol, promoted by Syngenta, was applied in an olive field in Spain. This project focuses on the study of the effect of the implementation of a more sustainable management measure such as the multifunctional inter-row cover crop on microbial biodiversity and soil health during nine months in three routine sampling times

throughout the year. The results indicate that no differences in alpha-diversity values were observed with the implementation of vegetation cover in an olive crop, neither at bacterial nor at fungal levels. However, differences in the bacterial and fungal population composition between the samples with and without inter-row cover crops were observed using beta diversity analysis, observing differentially enriched bacterial and fungal biomarkers when the vegetative cover was implemented. Finally, our results suggest that these changes in the soil microbiome conferring soil suppressiveness and antagonism to *Verticillium dahliae*, which is one of the most devastating fungi affecting the olive crop

Soil biodiversity, sustainable agriculture, soil health, microbial markers

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137. Genetic determinants of coumarin resistance in plant-associated *Pseudomonas*

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The soils around plant roots teem with millions of microbes, which form communities determining plant health status. Plants communicate and steer this microbiome to their benefit through metabolites exuded via their roots. Dicot plants can secrete fluorescent coumarins, for example, to alleviate Fe deficiency. Coumarins are known to be antimicrobial, and their presence can alter the rhizosphere microbiome composition of *Arabidopsis*. Specifically, the beneficial rhizobacterium *Pseudomonas simiae* WCS417 is resistant to the coumarin scopoletin, while certain soil-borne pathogens are susceptible. We hypothesize that the coumarin's reactive and antimicrobial nature limits colonization of susceptible microbes, while allowing resistant ones such as WCS417 to flourish. To verify this we want to discover genetic markers in sequenced *Pseudomonas* genomes involved in coumarin resistance using multiple approaches. First, we screened a collection of sequenced *Pseudomonas* for their resistance to coumarins scopoletin and fraxetin. We identified orthologous genes using Orthofinder, and linked their genomic presence or absence to the resistance levels of the *Pseudomonads* using Scoary2. We identified genes linked to fraxetin resistance, but not to scopoletin resistance. Since *Pseudomonads* are strongly resistant to scopoletin compared to other rhizobacteria, it is difficult to identify scopoletin resistance genes through comparative genomics. Additionally, we performed a Tn-seq experiment, where transposon mutant libraries of WCS417 were be exposed to different amounts of coumarins in liquid and solid cultures. After the sequencing, currently in progress, we can identify changes of the ratio of specific mutants after coumarin treatments, linking these mutations to susceptibility, and thus the genes to resistance. Considering the selective role of coumarins for beneficial microbes, this allows us to identify whether resistance genes and bacterial success in coumarin-rich environments are linked, potentially opening doors to new way of screenings bacteria for the construction of plant-beneficial inocula.

Coumarin, Resistance, *Pseudomonas*, Beneficial, Plant, Selection, GWAS, TnSeq

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138. THE APPLICATION OF RICE ENDOPHYTIC BACTERIA TO ARABIDOPSIS MODULATES ROOT PHENOTYPE AND PLANT GROWTH THROUGH MODIFICATION OF PHYTOHORMONES AVAILABILITY

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Microbiota provide important functions for plant performance, contributing to agro-system services. A molecular understanding of the interaction between plants and microbes can support the development of new microbial applications as an alternative to chemicals. A collection of putative endophytic bacteria previously isolated from rice (*Oryza sativa* L.) was tested for root phenotype alterations in vitro and plant growth-promoting activities in soil using *Arabidopsis thaliana*. Cocultivation of some bacteria strains with *Arabidopsis* plants showed an increase in primary and lateral root length due to direct contact, diffusible signals, and volatile compounds. The use of *Arabidopsis* fluorescent reporter lines for phytohormone signals revealed the activation of auxin and cytokinin-related pathways correlated with phenotypic characteristics. Subsequent application of bacteria to *Arabidopsis* seeds in soil showed a beneficial effect with an increase in the final foliar area. The use of plant probiotic bacteria has great potential, and plant microbiome-related technologies provide a functional complementarity for the discovery of molecular mechanisms responsible for beneficial traits.

Arabidopsis, biostimulants, root architecture, auxin, cytokinin

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139. Enhancing phosphate-solubilising microbial communities through artificial selection

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Microbial communities, acting as key drivers of ecosystem processes, harbour immense potential for sustainable agriculture practices. Phosphate-solubilising microorganisms, for example, can partially replace conventional phosphate fertilisers, which rely on finite resources. However, understanding the mechanisms and engineering efficient communities poses a significant challenge. In this study, we employ two artificial selection methods, environmental perturbation, and propagation, to construct phosphate-solubilising microbial communities. To assess trait transferability, we investigate the community performance in different media and a hydroponic system with *Chrysanthemum indicum*. Our findings reveal a distinct subset of phosphate-solubilising bacteria primarily dominated by *Klebsiella* and *Enterobacterales*. The propagated communities consistently demonstrate elevated levels of phosphate solubilisation, surpassing the starting soil community by 24.2% in activity. The increased activity of propagated communities remains consistent upon introduction into the hydroponic system. This study shows the efficacy of community-level artificial selection, particularly through propagation, as a tool for successfully modifying microbial communities to enhance phosphate solubilisation.

Phosphate-solubilising microbes, microbial communities, biofertilizers

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