Root-Benefit

Beneficial root-associated micro-
organisms for sustainable agriculture

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ROOT-BENEFIT Symposium & 3rd Annual Meeting, Granada 2026

BENEFICIAL MICROORGANISMS FOR SOIL HEALTH AND AGRICULTURE

Book of Abstracts

EEZ
ESTACIÓN EXPERIMENTAL DEL ZAIDÍN

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WELCOME

We are delighted to welcome you at the 3rd Annual Meeting of the COST Action ROOT-BENEFIT ‘Beneficial Microorganisms for Soil Health and Agriculture’. Set in the picturesque center EEZ-CSIC in the center of Granada. This year’s meeting will be attended by about 120 delegates representing 30 different countries. Eight invited keynotes at the forefront of research and development in both academia and industry, related to the 4 Working Groups of the Action, will spearhead the program. Those interventions will range from generating tools to predict plant-microbe interactions and designing synthetic microbial communities (SynComs) to strategies to promote the transition from the lab to the field. Also, we will have the presentation of the CAOS project, an outreach initiative to foster scientific vocations in secondary school students through the implementation of mini-research projects. A central theme of the meeting will be to unravel the molecular building blocks underpinning root beneficial microbes functioning and their use to promote the health of our soils, as well as more sustainable agriculture. Further 29 selected talks and 54 posters will complete a 3-day, discussion-packed and collaboration-fostering, program. We hope the meeting will facilitate interaction among experts from various research fields, including molecular biology, ecology, soil science, biological interactions, agriculture, and economics, with the aim of leveraging knowledge of beneficial root microbes for sustainable development.

We look forward to engaging with you all at the ROOT-BENEFIT 3rd Symposium

On behalf of the Organising Committee,

Benoit Lefebvre, Action leader.

The Conference Chairs:

Alfonso Clemente (Director of EEZ-CSIC)
Benoit Lefebvre (Action Chair, INRAE)
Juan A. Lopez-Raez (Local organizer, EEZ-CSIC)

MEET OUR KEYNOTE SPEAKERS

Carmen Escudero-Martinez (IRNASA-CSIC, Spain)

Dr. Escudero-Martinez is a junior Principal Investigator at the Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA) – Consejo Superior de Investigaciones Científicas (CSIC) in Spain. She moved in September 2025 with a competitive Talent Attraction Grant to start her lab. Prior to that, she had spent most of her career between the James Hutton Institute and the University of Dundee (UK), where she acquired expertise in Molecular, Biology, Microbial Ecology, and Genetics. Her research focuses on how plants shape microbial assembly and how microbial feedbacks influence nutrient acquisition and crop fitness, ultimately aiming to translate these insights to the field scale through the harnessing of microbial functional diversity.

Michael Schloter (TUM, Germany)

Prof. Schloter is professor of Environmental Microbiology at the Technical University of Munich (TUM) in Germany. He studied biology at the LMU from 1984 - 1989 and received his PhD from the University of Bayreuth in 1994. After several postdoctoral positions, he became head of the WG Microbial Ecology at the Institute of Soil Ecology of the GSF Res. Center in 2003. In 2009, the TUM appointed him honorary professor for Soil Microbiology. In 2011, Prof. Schloter became head of the Inst. of Comparative Microbiome Analysis at Helmholtz Zentrum München. In 2023, he was appointed to the professorship at TUM. Prof. Schloter has focused his research activities on the field of microbiome research. In particular, he is interested in the interaction of environmental microbiota with different host microbiomes, including the interaction with the human microbiome. He not only uses modern cultivation-independent "omics" based approaches to characterize microbiomes in their structure and function, but also tries to isolate important microorganisms and study their physiology in the laboratory.

Simona Radutiou (Aarhus University, Denmark)

Prof. Radutiou is Professor at the Department of Molecular Biology and Genetics at the Aarhus University. She leads a research group focused on studies of interactions established between plants and the large diversity of surrounding microbes. Her team uses genetic and molecular tools to decipher the role of plant components in establishing symbiotic associations with beneficial bacteria and fungi, or pathogenic associations with detrimental microorganisms. These interactions are studied by performing: i) targeted investigation of the mechanisms enabling plant LysM receptors to recognize these microbes at molecular, cellular and whole plant level, and ii) a broad investigation of microbiomes associated with different plant genotypes using next generation sequencing of microbial DNA. The aim of Prof. Radutiou is to use contrasting associations and microbial environments of increasing complexities (single microbes- tailored microbial consortia- soil complex microbiota) to understand how plants use their distinct genetic tools to select and accommodate beneficial microbes in their roots and rhizosphere. The long-term goal is to use our basic understanding to improve the ability of plant crops to select, associate and benefit from these microbial interactions in sustainable agriculture that limits the use of chemical pollutants.

Marc Ongena (Gembloux Agro-Bio Tech, Belgium)

Dr. Ongena got a PhD in Biochemistry at the University of Liège and did a Post-Doc at University Laval, Canada. He got a permanent position at the FRS-FNRS in 2007 and was promoted to Research Director in 2022. He is co-heading the Microbial Processes and Interactions Laboratory at TERRA Research Center and is currently leading a research group of 12 persons. His research work is dedicated to the study of soil dwelling Plant Beneficial Bacteria that retain a strong potential to protect plants against diseases and, therefore, represent promising alternatives to chemicals. He is investigating the molecular mechanisms underlying such biocontrol potential mainly based on a direct antagonistic activity toward pathogens and/or on the ability to trigger Induced Systemic Resistance (IR). This includes the study of the so-called bioactive secondary metabolites formed by these bacteria that are mostly responsible for biocontrol activity but also largely contribute to ecological fitness.

Tania Galindo (ETH Zurich, Switzerland)

Dr. Galindo is a researcher in the ‘Sustainable Agroecosystems Group’ at the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland). She studied Biology in Colombia and developed her PhD at the Pennsylvania State University (USA), working on the interaction between soil microbes and roots. Dr. Galindo is an expert on root biology and soil microbiology, having experience in managing transdisciplinary research projects in agricultural industry and academia. The goal of her current project MICROSEL is to find connections between plant adaptations and microbes for abiotic stress tolerance. The main goal of her research line is to help produce sustainable, biological-based solutions to plant health and fertilization.

Lena Neuenkamp (Bielefeld University, Germany)

Dr. Neuenkamp is junior professor and group leader of the biodiversity and ecosystem restoration group (BERG) at the Faculty of Biology of Bielefeld University (Germany). She did a PhD in Plant Ecology and Ecophysiology at the University of Tartu (Estonia). After several postdoc stages in Switzerland and Spain, she became Assistant Professor at the University of Munich in Germany in 2022. Since 2025, she is Junior Professor at Bielefeld University in Germany. Dr. Neuenkamp research is focused on plant-soil interactions, and ecosystem functioning. She is investigating the role of soil biota (such as mycorrhizal fungi) for plant diversity, ecosystem functioning, and ultimately the conservation and restoration of natural and urban ecosystems.

Marcel van der Heijden (University of Zurich; Agroscope, Switzerland)

Prof. van der Heijden is professor of Agroecology and Plant-Microbe Interactions at the University of Zurich (Switzerland) and Head of the research group Plant-Soil Interactions at the Swiss centre for excellence for agricultural research Agroscope. His research is focused on agro-ecosystems and crops, and study how plant microbiomes, mycorrhizal fungi and soil biodiversity influence plant growth and ecosystem functioning, bridging ‘Agroecology’ and ‘Plant-Microbiome Interactions’. The final goal of his research is to develop sustainable agro-ecosystems that are environmentally friendly and productive.

Elisa Pellegrino (Institute of Crop Science, Italy)

Dr. Pellegrino is Assistant Professor at the Institute of Crop Science at the Sant’ Anna School of Advances Studies in Pisa (Italy). Her research interests are Agroecology and Agrobiodiversity, Food Quality and Nutraceutics, and Sustainable Agricultural Systems. She studies soil quality and microbial diversity (fungi, bacteria and archaea) by using chemical, biochemical and molecular approaches. More specifically, she is interested in morphological, molecular and functional diversity of arbuscular mycorrhizal fungi (AMF), development of new molecular probes for classification/monitoring AMF, selection of exotic and native AMF isolates for inoculum production, setting-up field trials for the application of organic and biological fertilizers, and analyses of crop yield and quality.

ORAL PRESENTATIONS

WG1 session

Keynote talks

Carmen Escudero-Martinez (IRNASA-CSIC, Spain). Integrative crop trait profiling using combined root exudate and rhizosphere microbiota to identify traits that predict plant–microbe interactions

Authors: Carmen Escudero-Martinez, Eithne Y. Browne, Henning Schwalm, Michael Santangeli, Molly Brown, Lawrie Brown, David M. Roberts, Aoife M. Duff, Jenny Morris, Peter E. Hedley, Peter Thorpe, James Abbott, Fiona P. Brennan, Davide Bulgarelli, Tim S. George, Eva Oburger

The rhizosphere is defined as a resource exchange site essential for understanding plant adaptation to the environment. Plant-derived organic compounds released in the rhizosphere, through root exudates, nurture a distinct microbiota that enhances plant health and nutrition. Rhizosphere processes emerge from the interaction between the plant genome, the soil environment, and the resident microbiota, and can be regarded as genetically controlled rhizosphere traits. However, rhizosphere traits have been largely overlooked in crop breeding programs, representing the next frontier for sustainable agriculture, partly due to the challenges associated with their assessment, such as low throughput and the destructive nature of sampling.

Here, we developed and critically evaluated different sampling approaches for root exudates and rhizosphere microbiota from the same soil-grown plant, which increases the sampling throughput. Our results show that, under the tested conditions, different sampling approaches produced comparable microbiota and exudation patterns, enabling the integrated study of root exudation and microbial profiles from the same plant. Using this sampling approach, we identified high-throughput sampling crop traits that can predict changes in the rhizosphere and root microbiota composition of the climate-resilient crop barley [*Hordeum vulgare*] across genotypes and soil types. We identified root morphology parameters, classes of metabolites that impacted the microbiota composition in the root and/or the rhizosphere. Overall, we demonstrated that it is possible to use high-throughput sampling of crop traits that serve as proxies for predicting changes in rhizosphere processes.

Michael Schloter (TUM, Germany). Reprogramming the rhizosphere: Synthetic microbial communities to combat apple replant disease

Apple replant disease (ARD) is a major constraint for sustainable apple production worldwide, causing severe root damage, reduced plant growth, and significant economic losses in nurseries and orchards. The disease is associated with complex soilborne pathogen consortia and shifts in the rhizosphere microbiome, making targeted management strategies extremely challenging. Current control methods, such as soil fumigation, are increasingly restricted due to environmental and health concerns, creating an urgent need for innovative and sustainable solutions. Here we explore whether synthetic microbial communities (SynComs) can reprogram the rhizosphere microbiome and mitigate ARD.

Using metagenomic analyses of ARD-affected soils, we identified key functional deficits in microbial communities, particularly a reduced potential for degradation of aromatic compounds and plant-derived phenolics. Guided by these functional insights, we isolated candidate bacteria with complementary ecological traits, including *Rhodococcus pseudokoreensis* R79, capable of degrading phenolic compounds, and *Priestia megaterium* B1, a plant growth–promoting bacterium possessing genes for siderophore production, phosphate solubilization, and auxin biosynthesis.

Greenhouse inoculation experiments with apple plantlets grown in ARD soil revealed that *R. pseudokoreensis* R79 significantly altered rhizosphere community composition and increased bacterial diversity, while also influencing plant physiological parameters and phytoalexin profiles. In contrast, *P. megaterium* B1 transiently colonized apple roots and selectively enriched beneficial bacterial genera such as *Lysobacter*, *Pseudomonas*, and *Sphingomonas*. Field trials further demonstrated measurable improvements in plant performance, with yield increases of up to 25% under certain conditions. Our results demonstrate that rationally designed microbial inoculants can reshape rhizosphere microbial networks and partially restore functional traits lost in ARD soils. This work highlights the potential of microbiome engineering as a next-generation strategy to combat previously untreatable soil-borne plant diseases, paving the way for sustainable disease management in perennial cropping systems

CAOS Program. Francisco Martinez-Abarca (EEZ-CSIC, Spain)

The CAOS project is a scientific outreach Initiative promoted by research groups at the EEZ CSIC (Spanish National Research Council). Its objective is to foster scientific vocations in secondary school students by carrying out *mini*-research projects over 3-4 months during every school year. These projects on line with the different research lines of the groups encompass simple experiments (questions) in a variety of topics, ranging from: “soil legacy”, “antibiotics in soil”, “vermicomposting experiments” or “phenological studies of trees in different secondary schools”, etc. Each year's research culminates in a conference (200-300 attendees) where students present and share their results, experiences, and achievements. Simultaneously, a Scientific Publication Journal (High School Students for Agricultural Science Research – HSSASR) is generated with all communications. In such volume, within the scientific articles, students show their impressions, reflecting the impact of these projects on their learning. After nearly 15 years of success, the project seeks to export this model internationally through an Erasmus+ proposal call initiative with different groups belonging to ROOT-BENEFIT COST Action. The presentation will conclude with the participation of two students from previous editions who will illustrate the importance these research projects have had on their current academic decisions.

More information in: https://ucc.eez.csic.es/educacion-cientifica/high-school-students-for-agricultural-science-research/

O.1.1. Advances in endophytic microbial biostimulants for alfalfa (*Medicago sativa* L.) cultivation. Ismail Bezirganoğlu (Erzurum Technical University, Turkey).

Increasing climate change and drought stress severely threaten forage crop productivity and quality, consequently affecting livestock production. *Medicago sativa* (alfalfa) is particularly sensitive to drought, especially during early growth stages, where stress conditions reduce growth rate, photosynthetic capacity, water retention, and increase oxidative damage. This study aimed to evaluate the potential of drought-tolerant endophytic bacteria isolated from xerophytic plants to mitigate drought stress in alfalfa under greenhouse conditions. Within the framework of a completed TÜBİTAK 1002-A project (122Z810), 40 endophytic bacterial isolates were obtained from *Opuntia ficus-indica* and *Agave americana*. Following in vitro screening under PEG-6000-induced drought stress, four promising isolates were selected: *Bacillus wiedmannii* (A2), *Bacillus proteolyticus* (AT3), *Lysinibacillus macroides* (HT1), and *Enterobacter hormaechei* (H17). In the present study, single and combined bioformulations of these isolates were applied as foliar treatments to alfalfa (cv. Savaş) grown in non-sterile soil under controlled greenhouse drought conditions. Physiological and biochemical analyses were performed to assess plant responses. Results demonstrated that selected bioformulations significantly improved plant biomass, relative water content, and chlorophyll levels, while reducing lipid peroxidation and oxidative stress markers compared to non-inoculated stressed controls. These findings indicate that endophytic bacteria derived from drought-adapted plants represent promising biostimulant candidates for enhancing drought tolerance in alfalfa. This approach offers a sustainable strategy to improve forage production under water-limited conditions.

O.1.2. Towards a rice paddy microbiome atlas. Kevin Bretscher (Leiden University, The Netherlands).

Numerous studies have shown that soil and plant-associated microbiomes play an important role in plant health and especially in the response of plants to environmental stress. Rice (*Oryza Sativa* L.) is a globally important crop with economic and cultural significance which is affected by the effects of climate change, like salinization of fields. Despite great effort in studying rice microbiomes worldwide, we still lack clear answers on the microbial patterns in this important crop which are crucial to develop new biological solutions for agriculture. Here we processed 4127 16S rDNA samples from 958 locations covering 10 countries and multiple climate zones with a uniform pipeline. We used this dataset to compile a core microbiome for rice paddy soil, rhizosphere and root endosphere and defined taxa enriched in those compartments. We found associations between microbiome composition, pH, and salinity. Using different machine learning algorithms, we identified key biomarkers of alkaline and saline rice paddy soils using Shapley values. We also identified several important proteobacterial biomarkers, including *Pseudomonas* ASV143013, an ASV prevalent in saline rice paddies. Using our microbial isolate collection, we matched ASV143013 to *Pseudomonas* sp. MG15 isolated from a saline-alkaline rice paddy. Experimental validation of *Pseudomonas* sp. MG15 revealed its ability to alleviate salt stress in rice under saline irrigation. Our study shows how the application of explainable machine learning on public data can lead to identification of potentially beneficial microbes for agriculture and shows the importance of accessory microbiomes in plant stress alleviation.

O.1.3. Sunflower in contaminated soil "cry-for-help" of beneficial bacteria. Sara Borin (University of Milan, Italy).

In contaminated soils, beneficial interactions between plants and bacteria can drive the selection of holobionts that adapt as a whole to survive in the presence of pollutants. This study aimed to establish an effective rhizoremediation process in a soil impacted by petroleum hydrocarbons, utilizing a nature-based approach with significant potential for large-scale remediation. Rhizoremediation employs plants to stimulate the soil microbiome, encouraging the development of a microbial community in the rhizosphere that is enriched in pollutant-degrading bacteria, in turn reducing soil phytotoxicity. Root exudates are the primary factor shaping the rhizosphere microbiome, and their composition has been shown to shift under stress, functioning as a "cry-for-help" to attract beneficial microorganisms. By applying untargeted metabolomics, we found that sunflower alters its root exudation profile when exposed in vitro to xylene or when grown in contaminated soil. Some plant secondary metabolites that were significantly changed under pollutant stress enhanced the growth and rhizocompetence traits of selected hydrocarbon-degrading bacteria. These metabolites were further tested on the microbial cells directly detached from the sunflower rhizosphere using Hystodenz® density gradient centrifugation, showing biostimulant effect on dodecane degradation. Our findings demonstrate that sunflower actively recruits and stimulates beneficial bacteria through specific root exudates in response to contamination, highlighting the potential of root metabolomics analyses to improve rhizoremediation efficiency, paving the way for sustainable and effective strategies to restore polluted environments.

O.1.4. Leveraging lettuce genetic diversity to identify phosphate responsive root endophytes. Alessandro Ciampanelli (University of Turin, Italy).

Microbial-based approaches are increasingly proposed to reduce reliance on chemical fertilizers in agriculture. Among these, the most promising candidates are arbuscular mycorrhizal fungi (AMF), with their ability to extend the effective root absorption area and enhance phosphate uptake, and phosphate-solubilizing bacteria (PSB). However, their performance is often inconsistent and appears to depend on plant genetic diversity. With the aim of identifying genetic markers explaining plant differential responses to soil-beneficial microbes in crop species, a panel of 128 plant fully sequenced genotypes of *Lactuca sativa* was screened under controlled low-phosphate conditions using a defined synthetic microbial community (SynCom) with AMF and PSB. Lettuce genetic diversity had a strong impact on both physiological and morphological responses to inoculation, highlighting substantial genotype-by-microbe interactions. Profiling of root-associated microbial communities identified 22 amplicon sequence variants (ASVs) significantly associated with increased leaf phosphate content. Notably, 10 out of these ASVs belonged to the order Burkholderiales, known to be often associated with AMF, and Flavobacteriales. To move beyond correlation and test causality, a root-associated bacterial collection was established by isolating and culturing representative strains of the community profile from three different genotypes. Six isolates corresponding to ASVs linked to enhanced leaf phosphate content are now being evaluated in controlled inoculation assays to quantify their direct contributions to plant growth and phosphate nutritional state. This workflow connects host genomic variation, microbiota assembly and functional validation, supporting the development of genotype informed microbial inoculants for improved phosphorus use efficiency.

O.1.5. Waste organic amendments increased Ammonia Oxidizing Archaea (AOA) in the rhizosphere of grapevines. Giacomo Chiarelli (University of Bologna, Italy).

Nitrification is a vital biochemical process in agricultural ecosystems, responsible for converting ammonium into nitrate, more accessible for crops. Archaea are involved in the processes of soil nutrient transformation and contribute to maintaining ecosystem sustainability. In particular, ammonia-oxidizing archaea (AOA) are known as the dominant drivers in the soil nitrification process. The experiment was carried out in rhizobox, on 1 year-old vines of the cv. 'Sangiovese'. Rhizobox were filled with soil collected from a field trial where three types of amendments were applied since 2019 as follow: 1) mineral fertilization (MIN), according to the integrated crop management guidelines, which included 60 kg of N ha-1, 2) municipal organic waste compost (ACM), 3) agri-food organic waste compost (ACF), 4) defecation gypsum (GDD). The amendments were applied to return 240 kg N ha-1. Rhizobox were designed to collect the primary growing roots and their microbial community, rinsing the roots with a solution of magnesium sulphate. After 4 years of application, ACM, ACF and GDD increased N and C in the soil used, compared to mineral fertilizer (MIN). In particular, the use of ACF showed an increase of the total amount of Nitrososphaerota phylum and other N-fixing AOA in the rhizosphere of the plant treated with the ACF amended soil. The amount of nitrogen provided by the organic amendment treatments showed an increase of the archaeal population and abundance thus leading to higher levels of soil respiration and N fixation available for grapevine roots.

O.1.6. Decoding plant-microbiome interactions: insights into genetic players involved in beneficial microorganisms' recruitment in grapevine genotypes. Micol Guaschino (CREA-VE, Italy).

Understanding how grapevine rootstock genotype shapes microbiome assembly is essential for deciphering plant–microbe interactions and optimizing the recruitment of beneficial microorganisms. We characterized the root-associated microbiome of ten grapevine rootstocks (K5BB, Fercal, RDL, M4, 41B, RU140, 110R, 1103P, 420A, SO4) either inoculated with a synthetic microbial community (SynCom) or left untreated. Bacterial (16S) and fungal (ITS) communities were analyzed to identify the core microbiome and the dynamic fraction modulated by host-driven recruitment mechanisms. Alpha diversity analyses showed that bacterial richness and evenness were mainly driven by genotype, while treatment alone did not exert a significant overall effect. Post hoc comparisons revealed that certain genotypes displayed pronounced diversity shifts following SynCom application, suggesting genotype-dependent plasticity in microbial recruitment, whereas others showed limited responsiveness. Beta diversity further confirmed genotype as the primary factor structuring bacterial communities. A consistent pattern emerged for fungal communities at the genus level. Genotype explained the largest proportion of variance in community composition, whereas treatment effects, although statistically significant, accounted for a smaller fraction of variability and were strongly genotype-dependent. These results indicate that both bacterial and fungal microbiomes are predominantly shaped by host genotype, with SynCom effects contingent upon host genetic background. Transcriptomic and metabolomic profiling of selected genotypes is currently ongoing, and integrative analyses are being performed to uncover correlations between host gene expression, metabolic signatures, and microbiome composition. Moreover, selected contrasting genotypes have been crossed to generate a segregating F1 population that will be analyzed to dissect the genetic architecture underlying beneficial microorganism recruitment.

O.1.7. A multi-layer metagenomic framework uncovers a dominant *Acinetobacter* sp. driving tomato rhizosphere microbiota structure and functional potential. Alexandros Mosca (University of Catania, Italy).

Bacterial communities inhabiting the soil–root continuum play a crucial role in promoting plant growth and health. However, they may also act as reservoirs of antimicrobial resistance genes (ARGs), an emerging concern for human health. In this study, we explored the bacterial microbiota of tomato, a widely cultivated crop and relevant plant model whose functional microbial potential requires further investigation. Integrating amplicon and shotgun sequencing, we assessed the composition and functional potential of bacterial microbiota associated with two field-grown tomato genotypes ('Abbundo' and 'SV5T719') in the Mediterranean environment, alongside unplanted bulk soil. Amplicon sequencing revealed clear differentiation among the bulk soil, rhizosphere, root, and phyllosphere microbiota. Notably, an unexpected taxon identified as *Acinetobacter* accounted for more than 30% in the rhizosphere and roots of both genotypes. This led us to investigate 37 bulk soil and rhizosphere metagenomes, represented by 675 million reads and identified over 2,000 genes related to several plant growth-promoting traits, including nutrient mobilization for plant uptake. Interestingly, reconstruction of the 'resistome' revealed 25 ARGs, predominantly beta-lactamases. In parallel, we recovered a metagenome-assembled genome close phylogenetically to *Acinetobacter calcoaceticus*, whose putative beneficial functions related to phosphate solubilization, siderophore production, and reactive oxygen species detoxification were enriched, carrying a single ARG. An approach integrating two sequencing methods and specific repositories revealed a stress-alleviating bacterium dominating the root-soil interface, decoupled from ARGs and predominant over other microbes, which harbored more ARGs. These findings advance understanding of tomato microbiota to support sustainable agriculture while preventing risks within a One Health perspective.

WG2 session

Keynote talks

Simona Radutiou (Aarhus University, Denmark).

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

Marc Ongena (Gembloux, Belgium). 'Molecular basis of unsuspected mutualism between *Bacillus* and *Rhizophagus*'

In recent work, we have investigated the interaction between the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and the plant beneficial bacterium *Bacillus velezensis*. Our data unveil the rather unsuspected compatibility of this microbial interaction as *B. velezensis* is a strong producer of antifungal cyclic lipopeptides. The bacterium readily colonizes *R. irregularis* hyphae by forming strong biofilm expanding quite fast and uses fungal hyphae as highway for soil invasion and to colonize new plants. The bacterium does not negatively impact vitality and viability of the fungus and further investigation of the molecular aspects of this interaction revealed that such observed compatibility is due to a dampened production of fungitoxic fengycin. It also revealed that surfactin, another *Bacillus* lipopeptide, plays key roles in the chemical ecology of the interaction and that the microbial partnership enhances the systemic resistance of tomato against *Botrytis cinerea*. Beyond coexistence, these two plant beneficials thus establish a mutualistic relationship, which not only promotes their fitness but also leads to increased resistance of the host plant.

O.2.1. Cytokinins in and out - how ABC transporters may shape Medicago root morphology upon nitrogen deficiency. Michal Jasinski (Polish Academy of Sciences, Poland).

Despite numerous studies indicating a significant role of cytokinins (CKs) distribution for legume root morphology, our understanding of the underlying mechanism of CK transport remains limited. We have identified and characterized two *Medicago truncatula* full-size ATP-binding cassette (ABC) proteins of the G subfamily, namely ABCG40 and ABCG56, as CK transporters. Their expression is root-specific and is induced by nitrogen shortage. The MtABCG40 contributes to the horizontal translocation of CKs from xylem to the cells originating lateral organs. As such, it impacts root morphology, negatively affecting lateral roots and nodule formation upon nitrogen shortage. Moreover, the activity of this transporter may decrease the concentration of the apoplastic CKs in the root meristematic zone, reducing the sensitivity of the root apical meristem to CKs and primary root length. The MtABCG56 exports active CKs, produced in the epidermis in response to microsymbionts, to the cortex. Consequently, in this scenario, the CK signalling pathway is activated in the inner cortical layers, promoting cell divisions and the formation of root nodule primordia. Together, those transporters represent an example of functional specialization among members of this multigenic family and allow us to propose a model of CKs distribution in *M. truncatula* root.

O.2.2. The role of rhizobial volatile compounds in plant-microbe interactions. Maria J. Soto (EEZ-CSIC, Spain).

Bacteria emit a diverse array of volatile compounds that play important roles in both intermicrobial and interkingdom communication. In particular, volatile metabolites released by rhizobacteria can promote plant growth and enhance plant resistance to biotic and abiotic stresses. The term rhizobia refers to a specific group of rhizobacteria capable of inducing the formation of nitrogen-fixing nodules in legume plants. Despite their ecological and agricultural importance, very little is known about volatile compounds produced by rhizobia and their biological functions. During the study of rhizobial life on surfaces, we found that the *Medicago* spp. symbiont *Sinorhizobium meliloti* (Sm) releases several volatile compounds with notable biological activities. The volatile blend, or volatilome, emitted by Sm exerts phytostimulatory effects on the model plant *Arabidopsis thaliana*, significantly increasing both shoot and root biomass through a mechanism that is still under investigation. We also identified a volatile methylketone produced by Sm, 2-tridecanone, which functions as an infochemical that modulates key bacterial traits and hampers plant-bacteria interactions by interfering with microbial colonization of plant tissues. More recently, we showed for the first time that rhizobial volatile blends influence soil and plant microbiomes, and found that Sm volatilomes contribute to maintaining high abundance of *Sinorhizobium* populations within the Medicago root endosphere. Together, our findings highlight the important role of rhizobial volatile compounds in microbial communication and plant-bacteria interactions, opening new avenues for their application in sustainable agriculture.

O.2.3. Loss of Uridine diphosphate (UDP)-dependent glycosyltransferase gene UGT73F3 compromises root development and symbiotic performance in *Medicago truncatula*. Peter Kalo (HUN-REN Biological Research Centre, Hungary).

Legumes establish intimate endosymbioses with nitrogen-fixing rhizobia in root nodules and with arbuscular mycorrhizal fungi in roots. These interactions enhance plant growth by alleviating nitrogen and phosphorus limitation in nutrient-poor soils. Proper root development is essential for successful symbiosis, as defects such as impaired lateral root formation, shortened primary roots, or altered root hair density can severely compromise symbiotic establishment and function. We characterized the insertion mutant T0087 of *Medicago truncatula*, which exhibits pronounced defects in both root development and symbiotic organ formation due to disruption of the UDP-dependent glycosyltransferase gene UGT73F3. Mutant roots are significantly shorter than wild type, primarily because of accelerated cell differentiation and a reduced transition zone. Mitotic activity is decreased in both root and nodule meristems. Consistent with reduced cell division, nodule development is arrested, resulting in small, round, ineffective nodules. Members of the UGT73 family regulate secondary metabolism and stress responses. Metabolomic analyses revealed increased accumulation of the glycosylated flavonoid formononetin and an overall reduction in glycosylated triterpenoid saponins in the mutant. The severe phenotype of the *ugt73f3* mutant underscores the critical biological role of this enzyme. However, the mechanistic link between altered secondary metabolite glycosylation and reduced meristem activity remains unclear. UGT73F3 may function in glycosylation-mediated detoxification of toxic compounds such as saponins, or altered isoflavonoid glycoside levels may interfere with hormonal pathways regulating cell division in root and nodule meristems.

O.2.4. MicroRNA mediating multidirectional interaction of wheat with pathogenic *Fusarium culmorum* and beneficial *Trichoderma atroviride*. Lidia Błaszczyk (Polish Academy of Sciences, Poland).

Due to their existence in a complex environment and exposure to various microorganisms, plants have developed complex networks of gene regulatory pathways, key components of which are endogenous small

RNAs (sRNAs), including miRNAs. However, knowledge of the role of these molecules in the biotic interactions of common wheat with fungi is scarce. Therefore, the aim of this study was to understand miRNA-mediated communication between two wheat cultivars and the pathogenic species *Fusarium culmorum* and the symbiotic fungus *Trichoderma atroviride*. High-throughput sequencing of RNA, microRNA, and degradome was performed. Based on the RNA and microRNA sequencing data, differential analysis was performed, taking into account the wheat cultivar (Muszelka, Ambicja), its organ (leaf, root), phase of interaction with fungi (early, late), and treatment (pathogen, symbiont, pathogen/symbiont, water). Transcripts and miRNA molecules with significantly differential expression within the defined groups were identified. The detection of both plant- and fungal-derived miRNAs indicates a significant role for these molecules in the dialogue between wheat and both pathogenic and symbiotic fungi, as well as the three-way interaction between the studied organisms. This research was funded by the National Science Centre (project number 2022/47/B/NZ9/01282).

O.2.5. A novel tomato NAC transcription factor regulates lipid metabolism during microbe-induced resistance to herbivory.

Francisco J. Colina (EEZ-CSIC, Spain).

Microbe-induced resistance (MIR), triggered by root-associated beneficial microbes such as *Trichoderma* spp., enhances plant immunity through defense priming, making MIR a highly promising biotechnological tool for controlling crop pests. However, the molecular mechanisms underlying MIR remain poorly understood, limiting its predictability and broader application in agriculture. Using a combination of molecular, genomic, and metabolomic approaches, together with bioinformatic analyses and plant-microbe-insect bioassays, we investigated the function of a previously uncharacterized tomato NAC transcription factor (SlNAC) that is activated during *Trichoderma*-induced resistance to herbivory. Transcriptomic analyses revealed that SlNAC, a jasmonic acid (JA)-responsive factor, accumulates in leaves during MIR alongside JA-related transcripts. To assess its function, SlNAC knockout lines (*nac*) were evaluated in *Trichoderma*-tomato-*Manduca sexta* bioassays. In wild-type plants, fungal inoculation reduced larval survival, whereas this MIR-associated protection was abolished in *nac* mutants, demonstrating that SlNAC is required for the effective display of MIR. RNA-seq analyses revealed that *nac* mutants exhibit an altered transcriptional response to herbivory, affecting genes involved in lipid metabolism. DNA affinity purification sequencing (DAP-seq) identified direct SlNAC targets, and integration with RNA-seq data enabled reconstruction of a *SlNAC*-centered gene regulatory network, which included lipid-related targets. Together, our findings identify SlNAC as a key regulatory node for MIR, linking *Trichoderma*-mediated defense priming to a lipid transcriptional network underlying enhanced resistance to herbivory.

O.2.6. Effect of enhanced CO2 atmospheric levels in mycorrhiza colonisation and its role in plant defence.

Rosa Sanchez-Lucas (University of Birmingham, United Kingdom).

Forest trees have developed adaptive mechanisms over millions of years that enable them to withstand diverse biotic and abiotic stresses. However, climate change is intensifying these pressures, threatening forest ecosystems and their associated biodiversity. Rising atmospheric carbon dioxide (CO_2), a key resource for plant growth alongside water, represents a major driver of environmental change. While elevated CO_2 (eCO_2) can stimulate plant productivity, it may also induce physiological stress and alter plant interactions with other organisms. Previous studies have demonstrated that climate change factors, including warming temperatures, drought, and increasing CO_2 levels, significantly affect plant physiology and their relationships with symbiotic soil microorganisms such as mycorrhizal fungi. Mycorrhizal associations enhance plant nutrient uptake, growth, and resistance to environmental stresses. Despite their ecological importance, their role under future climate conditions remains poorly understood, particularly in field-based research. This study investigates the influence of mycorrhizal colonisation on oak seedlings, examines how eCO_2 affects fungal colonisation, and evaluates their combined impact on plant growth and defence trade-offs. Oak seedlings were grown under ambient and elevated CO_2 conditions at the Birmingham Institute of Forest Research Free Air CO_2 Enrichment (FACE) facility and subsequently infected with powdery mildew. Disease development was monitored from May to July. Root traits and mycorrhizal colonisation were assessed using ink-acid staining. Root metabolites were analysed through LC-MS/MS, with spectral processing performed using XCMS and statistical analyses conducted in MetaboAnalyst. Biomarker identification and pathway analysis were completed using MARVIS, alongside elemental carbon-to-nitrogen ratio measurements.

Pathways appearing as altered were related to phenylpropanoids and hormone signalling majorly. Results indicate that mycorrhizal associations contribute to disease resistance in oak seedlings, although this protective effect may decline under future elevated atmospheric CO_2 levels, potentially compromising forest health.

O.2.7. Time matters: mycorrhizal colonization dynamics shape plant interaction with parasitic nematodes.

Nicolas Marro (Institute of Botany-Czech Academy of Sciences, IBOT-CAS, Czech Republic).

Rhizosphere signalling molecules, such as the phytohormone strigolactones (SLs), are key plant-derived compounds that mediate associations with beneficial microorganisms, including arbuscular mycorrhizal fungi (AMF). Once AMF colonization reaches an ‘optimal’ level, plants downregulate SL production to prevent costly over-colonization. AM symbiosis provides plants with nutrients and protection by activating mycorrhizal-induced systemic resistance (MIR), for instance against plant-parasitic nematodes (PPN). Interestingly, PPN are also able to ‘sense’ SLs in the rhizosphere and be attracted by plant roots. Based on this evidence, we hypothesize that the moment of AM symbiosis establishment and PPN infection influence SL production, thereby affecting their own interactions. To test this, two experiments were performed under controlled conditions, using tomato as a model plant. The first experiment was conducted using a twin-chamber system, which allowed the assessment of PPN chemotaxis by the plant exudates. A non-mycorrhizal plant [No-Ri] was placed on one side, while the opposite side contained a plant inoculated with the AMF either previously [Ri1] or simultaneously [Ri2] infected with the PPN *Meloidogyne incognita*. The second experiment was conducted using individual pots to evaluate MIR and nematode performance under different mycorrhizal conditions (No-Ri, Ri1, and Ri2). We observed lower PPN attraction and performance in Ri1 than in Ri2, and transcriptional analyses suggest that this difference may be related to SL biosynthesis. We also detected higher MIR in Ri1, which may correlate with differential regulation of auxin metabolism. Together, these results suggest that pre-established mycorrhiza, but not simultaneous root colonization, reduces PPN attraction and activate plant defenses

WG3 session

Keynote talks

Tania Galindo (ETH Zurich, Switzerland). Synergies in the hidden half: root phenotypes as regulators of microbial ecosystem services.

Optimizing the root-soil-microbe interface is vital for transitioning agriculture toward higher resource efficiency. At the Sustainable Agroecosystems Group (SAE), we investigate how root systems capitalize on beneficial microbial associations under abiotic stress. I present a conceptual framework proposing that root architectural and anatomical phenotypes are not merely physical tools for soil exploration, but active regulators of microbial niches. Root ideotypes modify the rhizosphere via rhizodeposition, oxygenation, and nutrient depletion to recruit consortia that enhance resource acquisition. Building on this, we provide empirical evidence from spatial analyses demonstrating that root systems do not harbor a monolithic microbiome; communities are spatially structured by soil depth and root type. Specifically, Lateral Root Branching Density (LRBD) and Length emerge as significant drivers of microbial assembly. Finally, by integrating "Shovelomics" with metabarcoding, we reveal functional links between root-microbiome associations and crop performance. We found that anatomical phenotypes, including Number of Cortical Cell Files, Metaxylem Vessels, and Cortical Parenchyma Wall Width, significantly shape microbial communities. These configurations correlate with maize yield and domestication history, particularly under optimal plant growth conditions. Our findings suggest that phenotype-specific microbial associations drive soil processes in ways previously undiscovered. Breeding for specific root phenotypes offers a strategic pathway to harness synergistic microbial associations, providing a framework for the ecological and economic services of root-associated microbes.

Lena Neuenkamp (Bielefeld University, Germany). Leveraging root architecture and symbionts for ecosystem understanding and restoration

Restoring ecosystems requires more than reintroducing species—it demands an understanding of the processes that structure communities and sustain ecosystem service delivery. Trait-based ecology has provided powerful tools to link plant communities to ecosystem processes, yet it remains largely focused on aboveground characteristics. This perspective misses a critical dimension: the belowground arena, where roots and their microbial symbionts govern ecosystem processes, interactions, and community assembly—and thereby the delivery of key ecosystem services. In this keynote, I propose an integrated framework that connects above- and belowground trait dimensions. Root traits capture plant strategies of resource acquisition and cooperation, while simultaneously shaping the habitat and assembly of soil- and root-associated beneficial microbial communities, such as mycorrhizal fungi. By linking these perspectives, the framework provides a mechanistic basis to better understand how plant–soil interactions impact ecosystem processes and how this knowledge can inform ecosystem restoration.

Because most microbial symbionts interact with multiple plant species, the effects of plant–soil interactions on ecosystem processes are often most strongly expressed through their influence on plant community structure and assembly. Therefore, a key component of this framework is to link trait variation to community assembly processes, providing a valuable basis to evaluate the role of plant–soil interactions in ecosystem recovery and service delivery. Using this framework, I will outline key decision points for restoring and managing ecosystems: identifying disrupted above- and belowground assembly processes based on trait syndromes, and selecting restoration strategies that foster beneficial plant–microbe interactions.

O.3.1. Arbuscular Mycorrhizal Fungi (AMF) can overcome phosphorus deficiency and increase antioxidant activity and yield in lettuce. Fatjon Cela (University of Pisa, Italy).

Lettuce is an important economic crop widely used for its healthy properties, and it is of interest to increase them with minimal environmental impact. The purpose of this study was to evaluate the effect of the Arbuscular Mycorrhizal Fungus (AMF) *Funneliformis mosseae* in lettuce plants (*Lactuca sativa* L. cv. Salinas) cultivated with sub-optimal phosphorus (P) compared with non-inoculated controls at two different P concentrations. Results show that lettuce inoculation with the selected AMF can improve the growth and the nutritional quality of lettuce even at sub-optimal P. The leaf content of chlorophylls, carotenoids, and phenols, known as important bioactive compounds for human health, was higher in mycorrhizal lettuce plants compared with non-mycorrhizal plants. The antioxidant capacity of AMF plants showed greater values compared with control plants grown at the optimal P nutrition level. Moreover, leaf gas exchanges were higher in inoculated plants than in non-inoculated ones. Nitrogen, phosphorus, and magnesium leaf content was significantly higher in mycorrhizal plants compared with non-mycorrhizal plants grown with the same P level. These results imply that the AMF can promote plant growth, enhancing the nutritional value of lettuce even when it is cultivated with less P content, which can have positive ecological impacts on future cultivations.

O.3.2. Compost and vermicompost improve symbiotic nitrogen fixation, physiology and yield of the *Rhizobium*-legume symbiosis. German Tortosa Muñoz (EEZ-CSIC, Spain)

Compost and vermicompost constitute important sources of organic matter, nutrients, and beneficial microorganisms for agricultural systems. Their application improves soil physical and chemical properties while enhancing biological activity, including key plant–microorganism interactions. Among these interactions, the symbiosis between legumes and nitrogen-fixing rhizobia plays a central role in sustainable nutrient management. To evaluate the influence of compost and vermicompost on symbiotic nitrogen fixation, a systematic review of the scientific literature was conducted, followed by scientometric and meta-analytical approaches. The collected data were analysed in terms of variance, effect size, and percentage change relative to non-amended control treatments. Scientometric analysis identified several emerging and promising research areas, including the combined effects of compost or vermicompost with rhizobial inoculation on plant physiology, nitrogen fixation efficiency, soil quality, microbial diversity, economic performance, and tolerance to salinity stress. The combined use of compost and biochar was identified as a particularly recent and growing research trend. Additional relevant topics included the economic and environmental sustainability benefits associated with compost application in legume-based cropping systems. Meta-analysis results demonstrated that compost application increased nodule number by an average of 66%, nodule fresh weight by 52%, plant biomass by 48%, plant height by 21%, and yield by 20%, compared with untreated controls. Vermicompost generally produced even greater improvements across these parameters. Despite these positive effects, several knowledge gaps remain, including the role of compost in promoting nodulation under microbial inoculation, the interactive effects of compost and biochar on legume nodulation, and the influence of compost chemical and biological characteristics, particularly nitrogen content and native nitrogen-fixing microorganisms, on rhizobium–legume symbiosis. Overall, the findings confirm that compost and vermicompost are effective tools for enhancing soil fertility, legume productivity, and agricultural sustainability.

O.3.3. Grapevine physiology facing virus stress: Arbuscular mycorrhiza on the front line. Emanuel Gaši (Institute for Adriatic Crops and Karst Reclamation, Croatia)

Viruses represent a major hurdle to grapevine productivity, often resulting in a reduced lifespan and compromised fruit yield. Arbuscular mycorrhizal fungi (AMF), are able to confer many beneficial services to their plant partner including enhanced resilience to biotic stress. However, the impact of AMF on modifying the grapevine physiological response to virus infection is under investigated. In this study, we utilized AMF colonization of virus-infected grapevine to study its physiological changes and defence-related mechanisms. For this purpose, a two-year greenhouse experiment on grapevine (cv. Merlot) was set up with three types of AMF and five types of virus inocula. AMF inocula were *Rhizophagus irregularis* (RI) and *R. irregularis*, *Funneliformis mosseae* and *F. caledonium* (MIX), and virus inocula were different combinations of rupestris stem-pitting associated virus (R), leafroll-associated virus 3 (L) and pinot gris virus (P), with appropriate controls. By integrating phytohormonal profile, oxidative stress, photosynthesis-related parameters and related gene expression with virus and AMF quantification, we assessed the extent of this interplay on the grapevine host. The results showed distinct physiological changes in both years, with the treatment containing all three viruses (RLP) and MIX mycorrhiza showing the most prominent phytohormonal changes. Additionally, AMF plants reduced oxidative stress and showed overall increases in photosynthesis-related parameters and growth. The results aim to clarify the ecological service provided by AMF under biotic stress conditions, aligning with the WG3 objectives of understanding root–microbe benefits. Our findings may support strategies for leveraging beneficial microorganisms to enhance grapevine resilience.

O.3.4. Engineering hyphosphere microbiomes to enhance plant nitrogen acquisition via arbuscular mycorrhizal fungi. Valeriia Belova (Czech Academy of Sciences, Czech Republic).

Arbuscular mycorrhizal fungi (AMF) form a key group of plant symbionts responsible for a significant share of terrestrial nutrient fluxes. However, their limited lytic exoenzyme production prevents direct degradation of biopolymers. Thus, AMF depend on cooperation with bacterial communities in their hyphosphere, the thin soil layer surrounding their hyphae, to release organically bound nutrients, such as nitrogen (N) from chitin. Despite the potential of these interactions to improve plant mineral nutrition through AMF, our knowledge of hyphospheric bacteria, their interactions with AMF, and their cooperation in nutrient mobilization remains limited. To address this, three synthetic communities (SynComs) were assembled, each comprising three functionally characterized bacterial strains previously isolated from the AMF hyphosphere. The behavior of each SynCom was then assessed in the presence of AMF and 15N-enriched chitin in artificial soil in vitro. Bacterial community size, composition, and temporal dynamics were assessed by qPCR and NGS, while Isotope-Ratio Mass Spectrometry traced the N fluxes. Although community analyses are still ongoing, the isotopic data showed noticeable differences in N flux depending on the SynCom composition. Among the three tested communities, one (including a chitinolytic Streptomyces strain) showed a more pronounced positive effect on 15N release and transfer than another SynCom with the same Streptomyces strain but different companions, including the AMF sporulation-inducing bacterial strain. Hence, it seems that the microbial environment strongly shapes the function of fungal-bacterial interactions in the hyphosphere, thus offering unexpected but highly relevant insights into these beneficial interactions.

O.3.5. Soil Health and One Health implications in the Anthropocene: A perspective on microbial biotechnology. Salme Timmusk (Swedish University of Agricultural Sciences, Sweden).

Current trends in crop research focus on the challenges in future climates and the non-sustainable ecological footprints of food production systems identifying the links between microbial communities, crop and ecosystem services. Soils support terrestrial life by regulating ecosystem productivity, biogeochemical cycling, and exposure pathways that influence the health of humans, animals, and plants, collectively referred to as One Health. It is an integrated framework that recognizes the interdependence between human, animal, and environmental health. We need to continuously develop new knowledge about the vulnerability of agriculture and strategies to manage and prevent the consequences of various extreme weather impacts on soils, crops, animals and people. Although climate change is almost universally recognized, our understanding of its effects remains fragmentary, making coordination of management strategies difficult. Global changes have direct and indirect consequences for One Health. Microbiomes have significant potential for resilient, sustainable ecosystems and have been identified as a key research priority for soil health/One Health strategies [1]. While microbial biotechnology has the potential to restore soils, protect crops, and enhance One Health, microbiomes have traditionally been studied mostly within a single ecosystem. Ecosystems are interconnected and can influence one another; therefore, a holistic approach is essential to realize the potential and impact of technologies that address soil, plant, animal, and human health, as well as environmental issues. Reference 1. Timmusk S, Pall T, Raz S, Fetsiukh A, Nevo E. The potential for plant growth-promoting bacteria to impact crop productivity in future agricultural systems is linked to understanding the principles of microbial ecology. [1] Frontiers in Microbiology. 2023;14:1141862. doi: 10.3389/fmicb.2023.1141862. PubMed PMID: 37275175; PubMed Central PMCID: PMCPMC10235605.

O.3.6. Arbuscular mycorrhizal symbiosis regulates nitrification and enhances nitrogen retention across soil contexts. Anukool Vaishnav (Czech Academy of Sciences, Czech Republic).

Author(s): Anukool Vaishnav, Martin Rozmoš, Michala Kotianová, Hana Hršelová, Petra Bukovská, Jan Jansa

Arbuscular mycorrhizal fungi (AMF) form extensive hyphal networks that extend plant influence into bulk soil and enhance nutrient acquisition by host roots. Through several complementary experiments, we investigated how AMF regulate nitrogen (N) cycling across contrasting soil conditions and how these processes influence plant N uptake. Using 50 agricultural soils, we found that actively growing hyphae of *Rhizophagus irregularis* consistently suppressed ammonia oxidizing bacteria and comammox *Nitrospira*, while ammonia oxidizing archaea remained largely unaffected. This selective suppression was accompanied by shifts in community structure and correlated with soil pH. Contrary to expectations of direct competition for ammonium, AMF presence increased soil NH4+ concentrations, suggesting that mycorrhizal networks alter N transformations beyond simple substrate competition, potentially through modulation of mineralization dynamics or biological nitrification inhibition within the hyphosphere. To test the context dependency of these effects, we conducted another factorial experiment combining AMF inoculation, application of synthetic nitrification inhibitors, and soils differing in pH. While soil pH and inhibitor treatments strongly structured nitrification gene abundances, AMF altered N transfer from root-free soil to plants. Under non-mycorrhizal conditions, amoA gene abundance of ammonia oxidizing bacteria was positively associated with nitrate formation and 15N uptake from bulk soil. This relationship weakened in the presence of AMF hyphae, consistent with enhanced mycorrhiza mediated N acquisition. Together, these findings demonstrate that AMF symbiosis selectively regulates nitrifier guilds, modifies soil N fluxes, and enhances N retention. This work further highlights the potential of AMF to improve N acquisition efficiency and reduce losses in agroecosystems.

O.3.7. Capital allocation and financial sustainability of regenerative agriculture systems: Economic valuation of plant-soil-microbiome interactions under the EU Green Deal. Zoran Mastilo (University of East Sarajevo, Bosnia and Herzegovina).

Regenerative agriculture is increasingly recognized as a strategic pillar of the European Green Deal and sustainable food systems transformation. However, despite strong ecological evidence supporting the role of plant–soil–microbiome interactions in enhancing soil fertility, carbon sequestration, and long-term productivity, financial markets still face significant challenges in accurately valuing these ecosystem services.

This study develops an integrated financial–ecological framework for evaluating capital allocation efficiency in regenerative agriculture systems. By combining ESG investment principles, cost–benefit analysis, and sustainability-adjusted return metrics, the research examines how soil microbiome enhancement translates into long-term economic resilience and risk reduction. Particular emphasis is placed on the monetization of soil health improvements, reduced input dependency, and climate-related risk mitigation. Using panel data from EU agricultural systems and sustainability reporting standards, the study applies econometric modelling to assess the relationship between regenerative practices and financial performance indicators. The findings suggest that investments in microbiome-driven soil regeneration generate positive externalities that remain undervalued within conventional financial frameworks. The paper contributes to bridging the gap between ecological science and financial economics by proposing policy-aligned valuation models capable of supporting green capital flows toward regenerative farming. The results provide implications for policymakers, financial institutions, and investors seeking to align agricultural financing mechanisms with sustainability objectives.

WG4 session

Keynote talks

Marcel van der Heijden (University of Zurich; Agroscope, Switzerland). Mycorrhiza for sustainable plant production

Microbes play a key role in ecosystems. In recent years, we have been testing whether it is possible to engineer the microbiome in agricultural fields. We performed large scale field inoculations with arbuscular mycorrhizal fungi (AMF), a group of beneficial soil fungi that can improve plant nutrient uptake. We observed that field inoculation with AMF can promote plant yield (significant yield increases of up to +40% were detected). AMF inoculation was especially successful in fields where pathogenic fungi were present indicating that AMF enhance soil health. We now developed and tested practical tools (mycorrhizal granules) for large scale agricultural application. First results are promising. Moreover, we explore factors that determine AMF functioning and abundance in field soil. We observed that pesticides have a negative impact on AMF. Moreover, we also observed that the functioning of mycorrhizal communities in field soil depends heavily on which AMF species are present. Soil in which one particular AMF was present, were well adapted to forage for P, while in other soils without this one fungus, P foraging ability was poor. Overall, this talk highlights that AMF play a key role in agricultural systems and we demonstrate that field inoculation can significantly enhance plant yield.

Elisa Pellegrino (Institute of Crop Science, Italy). Field performance of native and exotic AMF consortia: implications for agroecosystem functioning and microbial networks

Although arbuscular mycorrhizal fungi (AMF) are increasingly applied in agriculture, their functional role under field conditions remains context-dependent and not fully understood. This study investigates the performance of native and exotic AMF consortia across Mediterranean agroecosystems, focusing on their effects on crop productivity, quality, and soil processes. A model framework was developed to identify efficient native AMF based on relationships among infectivity, effectiveness, soil properties, and plant diversity. Field trials on crops such as wheat, barley, and sunflower highlighted the importance of genotype and environmental conditions in shaping mycorrhizal outcomes. Beyond yield, AMF influenced product quality traits and soil nutrient dynamics. Metagenomic analyses revealed that crop benefits were associated with the proliferation of specific native AMF taxa, while exotic inocula variably altered resident microbial communities. Overall, results support the targeted use of native AMF consortia to enhance agroecosystem functioning, reduce ecological risks, and optimize microbial interaction networks under field conditions.

O.4.1. Beyond crop yield and nutrition: can mycorrhizal fungi contribute to biofortification? Stephanie Watts-Fawkes (Wageningen University, The Netherlands).

Zinc (Zn) deficiency is widespread throughout the world's agricultural soils leading to deficiencies in this essential micronutrient in cereal grains, and ultimately in the humans who rely on cereal-based diets. Arbuscular mycorrhizal (AM) fungi can acquire Zn for cereal and legume crops, so have been proposed as a biofortification tool. However, AM fungi also take up vast amounts of phosphorus (P) into the plant leading to greater accumulation of grain phytate; phytate is an "anti-nutrient" which chelates Zn in the human gut. We conducted experiments with nine different cereal and legume crops including rice, bread wheat and lentil, with or without inoculation with *Rhizophagus irregularis*. We grew the plants to maturity, then determined how inoculation with R. irregularis affected the grain Zn and phytate accumulation. From these measurements, we estimated the effect of *R. irregularis* on the bioavailability of Zn for human nutrition. There was substantial variation in response to inoculation with *R. irregularis*, both between crop species and between genotypes and varieties of the same species. AM fungal inoculation led to increased grain yield in some crops (sorghum, lentil), but in other crops, the additional P was stored in the grain as phytate, and AM inoculation led to much lower Zn bioavailability (rice, barley). The effect of AM fungal inoculation on the bioavailability of Zn in grain crops was highly dependent on the crop species in question. AM fungi may contribute to crop biofortification in some crops, but for others the benefit is likely minimal.

O.4.2. Ethical and safety aspects in the research of beneficial microorganisms for soil health and agriculture. Serghei Sprincean (Moldava State University, Moldova).

Research on beneficial microorganisms for soil health and agriculture offers promising solutions for sustainable food production, but it must be guided by strong ethical principles and human safety standards. Scientists study organisms such as nitrogen-fixing bacteria, mycorrhizal fungi, and other plant growth, promoting microbes to reduce reliance on chemical fertilizers and pesticides. Ethically, such research supports environmental sustainability and global food security. However, researchers have a responsibility to ensure that innovation does not compromise ecological balance or human well-being. Human safety is a central concern during laboratory research, field trials, and commercialization. Strict biosafety protocols are necessary to prevent accidental exposure, contamination, or unintended release of microorganisms. Even naturally occurring microbes can pose risks if handled improperly or if opportunistic pathogenic strains are involved. Laboratories must follow established biosafety guidelines, maintain sterile procedures, and conduct comprehensive toxicity and pathogenicity assessments before field application. Ethical considerations also include transparency, informed consent, and community engagement, particularly when field trials are conducted on farmers' land. Researchers must communicate potential benefits and risks clearly, ensuring that participants understand the scope of the study. Data integrity and honest reporting of results are equally important to avoid misleading stakeholders about effectiveness or safety. In cases involving genetically modified microorganisms, additional ethical scrutiny is required. Long-term environmental monitoring and regulatory approval processes help minimize unintended consequences. Also, responsible research practices, regulatory compliance, and respect for public health and environmental integrity ensure that beneficial microorganisms can be developed safely and ethically for sustainable agricultural advancement.

O.4.3. Mycorrhiza Induced Resistance: Functioning and applications in agriculture. Maria J. Pozo (EEZ-CSIC, Spain).

The mutualistic association between arbuscular mycorrhizal (AM) fungi and plants is widespread in nature and confers multiple benefits to hosts and ecosystems. Beyond improved nutrient acquisition, they enhance resistance/tolerance to biotic and abiotic stresses through greater metabolic and phenotypic plasticity, enabling more efficient activation of defenses. Numerous examples show AM plants exhibit increased resistance to pathogens and pests, a phenomenon known as Mycorrhiza-Induced Resistance (MIR). AM inoculation can affect not only the host plant but also the rhizosphere microbiota and even the microbiomes of attacking herbivores. Using tomato as a model, we show how MIR efficiently reduces damage by the destructive pest *Tuta absoluta*. We employ pharmacological and genetic approaches, omics technologies, and network analyses to understand the regulation and function of MIR and its effects across higher trophic levels, exploring its impact on other biocontrol agents. The strategy allowed the discovery of bioactive compounds mediating these effects with potential for biotechnological applications. Proof-of-concept field experiments under agronomic conditions show that MIR can reduce pest incidence without yield penalties, and modulate plant secondary metabolism improving fruit quality and antioxidant properties. We discuss the opportunities and challenges of integrating AM inoculants into integrated pest management programs for sustainable crop protection and fruit biofortification. Lidoy J *et al,* 2025. J Agric Food Chem, doi: 10.1021/acs.jafc.5c14198. Minchev Z *et al,* 2024. J. Pest Sci, doi: 10.1007/s10340-025-01966-3 Minchev Z *et al,* 2024. Agron Sustain Dev, doi:10.1007/s13593-024-00991-3 Fiorilli V *et al.,* 2024. Ann Rev Phytopathol doi: 10.1146/annurev-phyto-121423-042014.

O.4.4. Nature knows first: Learning from tomato exudates to build better SynComs. Juan Ignacio Vilchez (ITQB-NOVA, Portugal).

Plant root exudates are key determinants of rhizosphere assembly, particularly under abiotic stress. We hypothesized that stress-specific exudation patterns define selective microbial recruitment and can therefore serve as a blueprint for rational synthetic community (SynCom) design. Tomato plants were grown under control, salinity, and drought conditions, and root exudates were collected to characterize stress-dependent shifts in rhizosphere recruitment dynamics. Distinct exudation signatures were associated with each environmental condition and correlated with the enrichment of specific microbial taxa, supporting the concept that plants actively steer microbiome assembly through context-dependent chemical signaling. Based on these recruitment patterns, we designed condition-tailored SynComs composed of strains preferentially enriched under each stress regime. Application of these exudate-informed SynComs significantly enhanced early-stage tomato growth under their corresponding stress conditions, demonstrating improved establishment and biomass accumulation. Beyond SynCom design, our findings suggest that exudate-derived signals themselves could be leveraged to modulate resident soil microbiomes prior to stress exposure. Such signal-based strategies may enable microbiome steering in a prophylactic manner, reinforcing beneficial microbial networks before adverse conditions arise. Together, our results provide a framework for ecologically informed microbiome engineering that integrates stress-dependent plant signaling into the development of context-specific interventions aimed at improving crop performance under environmental stress.

O.4.5. Pathogen infection and mycorrhizal inoculation differentially shape the root-associated microbiome of *Prunus* rootstocks with contrasting susceptibility to white root rot disease. Amaia Nogales (IRTA, Spain).

Armillaria mellea is a soil-borne fungal pathogen causing white root rot (WRR) disease, a major constraint for almond production in Mediterranean regions. Because chemical treatments are ineffective and no *Prunus* rootstock is fully resistant, understanding mechanisms underlying partial tolerance becomes essential. One factor potentially contributing to this tolerance is the composition and responsiveness of the root associated microbiome. This study analyzed plant performance and root microbiome shifts in response to an arbuscular mycorrhizal fungus (AMF) and *A. mellea* inoculations (single and combined), in two *Prunus* rootstocks with contrasting WRR susceptibility—Rootpac 40 (R40) and Rootpac R (RR)—grown for 11 months in potted soil from an almond orchard. Both *A. mellea* and AMF inoculations reduced fungal alpha diversity in both rootstocks. R40 was highly susceptible to *A. mellea*, but AMF inoculation reduced mortality and symptom severity. In pathogen inoculated plants, Streptomyces was significantly depleted and Hyalorbilia was enriched in the root associated community, whereas AMF + *A. mellea* co-inoculation promoted enrichment of potentially beneficial bacteria such as *Streptomyces* and the *Rhizobium* complex. RR showed no mortality and no growth response to AMF or *A. mellea*. Interestingly, *A. mellea* inoculation led to enrichment of potentially beneficial genera such as *Nocardioides*, *Hyalorbilia* and *Trichoderma*, while AMF + *A. mellea* co-inoculated plants additionally showed enrichment of the *Rhizobium* complex. Overall, these findings demonstrate that host genotype strongly influences microbial recruitment and that targeted microbial inoculations can induce beneficial shifts in the root microbiome that may contribute to WRRD tolerance, supporting microbiome informed approaches to enhance rootstock resilience.

O.4.6. Microorganisms registerability: how to transform EU agriculture. Jose Carvalho (Certis, Portugal).

There is exciting innovation in R&D labs which could boost the use of microorganisms in agriculture, whose benefits are unquestionable. However, their use is still limited as the rules for product development and registrability have been limiting innovation and the widespread use of microorganisms. Looking at these regulatory and administrative barriers – most of them due to historical and policy reasons within the EU, and no scientific reasoning – is crucial to unlock innovation in Europe and ensure growers have access to the benefits produced by the research networks on microorganisms in agriculture. This talk will discuss the existing gaps between research and development to field use, and the actual challenges in delivering microorganism(s) products for agriculture use. Being aware of the current roadblocks is essential to enable sensible proposals to remove them from the road and deliver innovative solutions for European growers. Such proposals can only succeed if all actors involved in this transition process have a common understanding and an open dialogue concerning the existing barriers and challenges to be overcome, from the early stage of research until post market authorization of microorganism-based products.

O.4.7. Arbuscular mycorrhizal fungi and dark septate endophyte dual inoculation shapes grapevine physiological responses and root carbon exudation under drought stress. Lucas Marginedas (IRTA, Spain).

Dark septate endophytes (DSE) are melanized fungal endophytes that colonize roots and can provide beneficial effects to plants in enhancing tolerance to drought. Coexistence of DSE and arbuscular mycorrhizal fungi (AMF) is common in roots, but their combined inoculation has not been evaluated in grapevine. Our aim was to assess the effects of single and dual inoculation of the AMF *Rhizophagus irregularis* (Ri) and the DSE Aureobasidium pullulans (Ap) in two grapevine rootstocks (Folla Redonda-FR; Richter 110-R110) under optimal (I-100%) and deficit irrigation (I-50%) conditions. Five months after inoculation, we measured photosynthetic rate (A), shoot length (SL), root biomass (RB), and total organic carbon (TOC) root exudation. In FR, I-50% led to a decrease in RB, but Ri-inoculation had a positive effect on RB and A under both irrigation regimes, and on SL under I-100%. However, Ap-inoculation did not lead to growth changes. Inoculation with either of the microorganisms reduced TOC exudation, whereas I-50% increased it. In R110, a negative effect of I-50% was found on SL, but single Ri-inoculation increased RB. Moreover, both Ri and Ap-inoculation enhanced significantly A with the effect of Ap being only significant at I-50%. On the other hand, single Ap-inoculated and dual-inoculated plants at I-50% had the lowest TOC exudation, but at I-100% highest TOC exudation was found in non-inoculated and dual-inoculated plants. Overall, dual inoculation appears to enhance drought tolerance in both rootstocks, while Ap alone modulates carbon exudation, which can have consequences for the rhizosphere microbiota and resource-use efficiency.

POSTER PRESENTATIONS

WG1 session

P.1.1. Identification and characterization of native PGPR strains isolated from the rhizosphere of common bean (*Phaseolus vulgaris* L.) cultivated in Erzurum province. Fatih Dadaşoğlu (Atatürk University, Turkey)

The rhizosphere is a critical microecosystem in which intensive interactions between plant roots and soil microorganisms shape plant growth and nutrient acquisition processes. Legume species, due to their rich rhizospheric microbial diversity, constitute an important ecological niche for plant growth-promoting rhizobacteria (PGPR). This study aimed to isolate and characterize bacterial strains exhibiting PGPR traits from the rhizosphere of common bean (*Phaseolus vulgaris* L.) cultivated in Erzurum province. A total of 19 distinct bacterial strains were isolated and purified from rhizosphere soil samples. The isolates were screened for key plant growth-promoting traits, including nitrogen fixation and phosphate solubilization capacity. Strains showing positive results for both traits were subjected to molecular identification through 16S rRNA gene sequencing followed by sequence-based taxonomic analysis. The findings revealed that among the 19 isolates, two strains demonstrating positive PGPR characteristics were identified as Rhizobium sp. These strains are considered promising candidates for potential application as PGPR agents in common bean cultivation. The identification of indigenous PGPR strains adapted to local agroecological conditions provides a scientific basis for the development of bioinoculants and contributes to sustainable bean production through the enhancement of root–microbe interactions.

P.1.2. Decoding the hidden secrets of plant microbiomes. Victor Carrion (Malaga University, Spain)

Plant roots harbour an extraordinary diversity of microorganisms, with cell densities often surpassing those of the plant itself. Studies have shown that the plant microbiome plays a key role in enhancing plant tolerance to both abiotic (e.g., drought) and biotic stresses (e.g., disease), while also contributing to nutrition, growth, and development [1]. However, the functions and mechanisms of most plant-associated microorganisms remain poorly understood. Recent advances in 'omics technologies have expanded our knowledge of microbiome diversity and function, shedding light on the complex interactions within plant-associated microbial communities. This presentation will provide new findings on the role of rhizosphere and endosphere bacteria in protecting plants from biotic and abiotic stresses. Additionally, we introduce bacLIFE [2], a novel bioinformatics tool designed for genome annotation, large-scale comparative genomics, and the prediction of lifestyle-associated genes (LAGs) in bacteria. Using bacLIFE, we analyzed 16,846 genomes from the *Burkholderia*/*Paraburkholderia* and *Pseudomonas* genera, identifying hundreds of genes potentially linked to plant pathogenic lifestyles. Site-directed mutagenesis and plant bioassays confirmed that six predicted LAGs are indeed involved in the phytopathogenic lifestyle, including genes encoding a glycosyltransferase, extracellular binding proteins, and homoserine dehydrogenases. An overview will be given on the wealth of genes and functions of the plant microbiome.

P.1.3. Exploring Industrial Soils for Fengycin- and Iturin-Producing Microorganisms with Agricultural Potential. Natalija Atanasova-Pancevska (Ss. Cyril and Methodius University in Skopje, North Macedonia)

Soil microorganisms play a central role in maintaining soil health and supporting sustainable agriculture through nutrient cycling, disease suppression, and production of bioactive compounds such as biosurfactants. In this study, soil samples collected near the “OKTA” Oil Refinery in Skopje were analyzed for their physical and chemical properties, showing an average pH of 7.63, redox potential of -27.3 mV, and organic carbon content of 0.135%. Enumeration of cultivable bacteria revealed an average of 1.68×10^6 CFU g^{-1}. Representative isolates were characterized via 16S rRNA gene sequencing, confirming them as *Delftia tsuruhatensis* and *Delftia lacustris*. These isolates were screened for biosurfactant production through oil spreading, parafilm and glass slide collapse assays, and emulsification activity over 216 hours. They exhibited strong biosurfactant activity, with oil spreading zones averaging 4.85 cm and early emulsification indices exceeding 50%, highlighting their potential for hydrocarbon dispersion and soil improvement. Molecular screening revealed the presence of fengycin and iturin genes, indicating the capacity to produce multiple bioactive lipopeptides with antifungal and emulsifying properties. These findings demonstrate the dual functionality of the isolates as agents for both soil health enhancement and biocontrol of phytopathogens. Furthermore, this study illustrates the potential of industrially influenced soils with distinct physicochemical characteristics as reservoirs for diverse, yet-to-be-discovered microorganisms with applications in sustainable agriculture and environmental management.

P.1.4. Endophytic bacteria as sustainable biocontrol agents in winter wheat (*Triticum aestivum* L.) in Lithuania. Daiva Burokiene (Nature Research Centre, Lithuania)

In Lithuania, winter wheat (*Triticum aestivum* L.) is often affected by major fungal pathogens that reduce yield and grain quality. The most common diseases include *Microdochium* spp., *Fusarium* spp., *Blumeria graminis f. sp. tritici*, *Puccinia* spp, *Zymoseptoria tritici*, *Pyrenophora tritici-repentis*. These diseases are promoted by humid and fluctuating climatic conditions, emphasizing the need for sustainable, biologically based management strategies. The intensive use of agrochemicals has mitigated some yield losses but has caused significant environmental and health concerns. Therefore, there is growing interest in sustainable alternatives that maintain productivity while reducing the use of chemicals. Plant growth-promoting bacteria (PGPB) are a promising solution. They enhance plant growth and resilience through biological nitrogen fixation, phytohormone production (auxins, cytokinins, gibberellins, abscisic acid, ethylene), phosphate solubilization, siderophore production, and induction of systemic resistance, and can suppress phytopathogens. In this study, 566 culturable endophytic bacterial isolates were obtained from surface-sterilized winter wheat roots collected in eastern Lithuania during the 2025 growing season. The isolates were evaluated for their plant growth-promoting potential and antifungal activity which were considered suitable candidates for integrated pathogen control in cereals. This research provides a basis for selecting effective endophytic bacteria that can simultaneously promote wheat growth and suppress major fungal pathogens, offering a practical approach for sustainable cereal production.

P.1.5. Induced resistance against Fusarium wilt of muskmelons using endophytic *Fusarium clavum* EeR24 from the Arava desert. Stanley Freeman (OB, Israel)

In the present study, 23 endophytic fungal isolates belonging to nine different genera were isolated from *Ecballium elaterium* and screened to determine antifungal potential against *Fusarium oxysporum f. sp. melonis* (race 1.2) (Fom) using an in-vitro antagonistic assay. Two endophytic isolates (*Fusarium* sp. EeR4 and *Fusarium clavum* EeR24) exhibited inhibitory effects against Fom on quarter strength PDA plates. In growth chamber experiments, *F. clavum* EeR24 colonized melon seedlings and significantly protected plants from wilting as compared to non-colonized pathogen-challenged seedlings. Under greenhouse conditions, *F. clavum* EeR24 significantly improved morphological and physiological traits including plant height, weight, number of leaves, membrane stability, photosynthesis, stomatal conductance and transpiration in *Cucumis melo*. Endophytic colonization improved catalase (56 %), guaiacol peroxide (47 %) and superoxide dismutase activity (25 %), flavonoid and phenolic content by 11-59 % compared to non-colonized Fom challenged plants. Lipid peroxidation significantly decreased by 37 % and proline accumulation increased by 70 % in colonized plants as compared to non-colonized plants. Histochemical analysis also indicated that endophytic colonization considerably reduced the levels of H2O2, O2-, malondialdehyde and cell mortality in Fom challenged plants. In addition, the culture filtrate and organic residues of *F. clavum* EeR24 inhibited the mycelial growth of Fom by 52-58%, respectively. Furthermore, a study on spatial colonization of the endophyte and the pathogen by using GFP and RFP-tagging indicated that both the endophyte and the pathogen simultaneously colonized the root tissues of *C. melo*, however, the endophyte significantly reduced pathogenicity of Fom. These results suggest that endophytic *F. clavum* EeR24 may be developed as an effective biocontrol agent for the management of Fusarium wilt in melon plants under field conditions.

P.1.6. Effects of nitrogen-fixing bacteria isolated from potato tubers and their EVs on in vitro potato growth. Gülce Güralp (Sabancı University, Turkey)

Potato (*Solanum tuberosum*) is the 4th most important crop in terms of fresh production and Türkiye is ranked the 13th largest producer in the world. In potato production, nitrogen is the yield limiting macronutrient due to its role in potato development and productivity. Beneficial microbes are viable alternatives to chemical fertilizers, generally used to meet the nitrogen requirement of plants. The study aims to explore the effects of nitrogen-fixing bacteria and their EVs on potato growth and to develop a potential biofertilizer using bacteria/EVs. EVs-released by nitrogen-fixing bacteria may carry genes/biomolecules related to nitrogen fixation to help potato growth and mediate relationship between the plant-endophyte symbiosis. Potato tubers were surface-sterilized and used to isolate culturable endophytic bacteria, which were collected from 15 high-yielding potato fields in 5 provinces of Türkiye. Pure bacterial isolates with nitrogen-fixing ability were screened by inoculating into nitrogen-free media. Selected isolates have been identified by 16S rRNA sequencing. The most common bacterial isolates across fields will be selected for EV production and EVs released by nitrogen-fixing bacteria will be characterized. An isolate identified as Pseudomonas strain was evaluated for EV production. This isolate and its EVs will be investigated for potato growth promotion by inoculating potato plantlets. Extensive bacterial isolates naturally found in potato will have been screened to identify significant strains for a potential biofertilizer that enhance nitrogen fixation in potato plants. Investigation of the effects of EVs-released by nitrogen-fixing bacteria will contribute novel insights into plant-microbe interactions. This study has been supported by TUBITAK, 124O159.

P.1.7. FoodN’Feed: Locally adapted beneficial microbiomes and synthetic communities for enhanced grain legume performance and sustainable protein production. Louise Mathier (Zurich University of Applied Sciences, Switzerland)

Europe’s increasing demand for sustainable, low emission protein sources highlights the importance of grain legumes, such as soybeans, faba beans, and peas. These crops depend on root associated beneficial microorganisms, particularly nitrogen fixing rhizobia, to reduce the need for synthetic fertilizers and support soil health. However, these interactions are underutilized in European agriculture, where commercial inoculants often perform inconsistently due to competition with well adapted native soil communities. The FoodN’Feed project aims to develop a locally adapted microbial resource base for major Swiss grain legumes. Through collaborations with the ROOT BENEFIT and MiCropBiomes COST Action, we will test their broader applicability across Central and Northern Europe. Our objectives are to: 1. Characterize the diversity and functional potential of rhizobial and non-rhizobial beneficial microbes in nodules, rhizosphere, and soils using 16S rRNA and ITS metabarcoding; 2. Identify true nodule forming partners through targeted sequencing of the nodD regulatory gene; 3. Recover high resolution genomic information from nodules via whole metagenomics to obtain metagenome assembled genomes (MAGs); 4. Isolate, identify, and sequence the genome of promising bacterial and fungal strains to build a curated culture collection; 5. Design and assemble synthetic communities (SynComs) of native, complementary microbes tailored to Swiss edaphic and climatic conditions; 6. Co evaluate the performance, stability, and transferability of these SynComs with partners in contrasting agro climatic environments. This project lays the foundation for robust, highly efficient microbial inoculants by integrating community profiling, genome-resolved analyses, and SynCom design. The anticipated impact includes reduced fertilizer dependency, improved legume performance, enhanced soil health, and greater regional protein self-sufficiency in the face of climate change.

P.1.8. Characterization of the rhizosphere microbiome from productive potato fields in Türkiye. Stuart Lucas (Sabanci University, Turkey)

Nitrogen is the most important yield limiting nutrient for potato cultivation. In Türkiye, most additional nutrients required by crops are supplied by chemical fertilizers, although various biofertilizer products are also available. Our aim is to identify beneficial bacteria that promote potato growth and nitrogen utilization from strains that occur naturally in Türkiye, with the goal of developing components for a native non-GMO biofertilizer. We anticipate that locally adapted, plant beneficial bacteria can be found in productive potato fields. Therefore, potato tuber, root and soil samples were collected from highly productive potato farms in 5 different provinces with high levels of potato production. The soil samples were analyzed for their physical and chemical characteristics and rhizospheric bacteria will be isolated from soil clinging to the surface of roots. Over 500 isolates of root-associated bacteria were sub-cultured, characterized by microbiological methods and screened for potential Nitrogen-fixing activity. From the same soil samples, total DNA was extracted and meta-barcoding analysis carried out using the 16S rRNA gene to characterize the bacterial microbiome; also, primers were designed to amplify the nifH and nifD genes, with the aim of assessing the prevalence and diversity of nitrogen-fixing micro-organisms in the rhizosphere. Using these approaches, a core microbiome was defined including genera that were present in all rhizospheric soil samples, and hub taxa that are expected to be involved in maintaining the microbiome community. These were compared with the cultured isolates to select strains to test for plant growth promoting activities.

P.1.9. Screening and functional characterization of nitrogen-fixing bacterial isolates from potato cultivation soils for sustainable agriculture. Sena Usta (Gebze Technical University, Turkey)

With the Green Revolution, the continuous increase in human population and food demand has transformed agriculture into global industry. As the fourth most important food crop worldwide, the potato (*Solanum tuberosum* L.) plays a vital role in global food security. To meet its high nitrogen requirements and ensure yield, chemical nitrogen fertilizers are extensively used; however, their excessive application leads to severe environmental degradation, including soil and water pollution. As a sustainable alternative, biofertilizers offer a promising solution to reduce reliance on chemical inputs while maintaining crop productivity. In this study, 45 soil samples were collected from the rhizosphere of potato plants across major potato-growing regions in Türkiye. From these samples, 542 bacterial colonies were isolated, and selected based on distinct morphological characteristics such as pigmentation, margin, texture, etc. The isolates were screened on nitrogen-free (NFB) medium to identify potential nitrogen-fixing bacteria, resulting in the selection of promising candidates as nitrogen fixers. These were further tested using Nessler's reagent to detect and quantify ammonium (NH_4^+) production, the 30 isolates that showed the highest ammonium levels were selected for detailed characterization. Biochemical tests were conducted to assess the metabolic properties of the selected isolates and 16S rRNA gene sequencing was carried out for these isolates. Based on the sequence analysis, some of the isolates were identified as belonging to *Pseudomonas* sp., *Acinetobacter* sp., and *Enterobacter* sp. which are known to inhabit plant rhizospheres and include strains with potential nitrogen-fixation and plant growth promoting traits, supporting their relevance to aims of the research project This study was supported by TÜBİTAK (124O159).

P.1.10. Edaphic context drives the citrus rhizosphere microbiome response to water stress. Helena Santiago Lima (Instituto Agronomico de Campinas, Brazil).

Soil microbial communities are key mediators of plant adaptation to water stress, promoting nutrient acquisition and supporting root performance. This function is particularly relevant for perennial crops such as citrus, which are exposed to recurrent seasonal fluctuations across multiple growing cycles. However, the extent to which soil properties, plant genotype, and water availability interact to shape rhizosphere bacterial communities remains unclear. We evaluated the impact of water stress on citrus rhizosphere bacterial communities under controlled greenhouse conditions, considering the combined influence of soil type and rootstock genotype. Two contrasting soils from São Paulo State (Brazil) were used: a drought-adapted soil from the northern region and a less drought-adapted soil from the southern region. A commercial pine bark-based substrate served as control. Plants were grafted onto two rootstocks differing in drought tolerance i.citrandarin C18 (*Citrus reticulata* × *Poncirus trifoliata*), more tolerant, and ii.citrandarin C299, less tolerant; and subjected to well-watered, drought-stressed, and rehydrated conditions over 42 days. Bacterial community composition was assessed via 16S rRNA amplicon sequencing. Soil type was the main driver of community structure ($R^2 = 0.39$, $p = 0.001$). Rootstock and water status alone had weak, non-significant effects. In contrast, their interactions were significant: Soil:Rootstock ($R^2 = 0.44$, $p = 0.001$), Soil:Stress ($R^2 = 0.43$, $p = 0.001$), and Soil:Rootstock:Stress ($R^2 = 0.51$, $p = 0.001$). These findings indicate that microbial responses to drought and plant genotype are strongly soil-dependent, reinforcing the need to consider local edaphic context when designing microbiome-based strategies for enhancing plant resilience.

WG2 session

P.2.1. Beyond the Symbiont: Mycorrhiza-Induced Resistance (MIR) and inter-plant signaling against *Tetranychus urticae*. Victor Flors (Universitat Jaume I, Spain)

The two-spotted spider mite, *Tetranychus urticae*, remains one of the most polyphagous and damaging pests in agriculture. Our current research study explores the role of arbuscular mycorrhizal fungi (AMF) in enhancing plant resilience through Mycorrhiza-Induced Resistance (MIR) in citrus and tomato models. Our findings demonstrate that mycorrhizal plants exhibit significantly higher resistance to *T. urticae* compared to non-colonized counterparts. This resistance stands on a multifaceted reprogramming of the plant's immune metabolism, characterized by the elevated production of secondary metabolites, specifically phenylpropanoids and flavonoids. Crucially, this defensive state is driven by the simultaneous activation of Jasmonic Acid (JA)-dependent pathways and Systemic Acquired Resistance (SAR) elements, providing a robust, dual-layered defense strategy. Furthermore, we show that MIR is not a localized phenomenon but extends to the plant community through volatile-mediated communication. Upon mite infestation, mycorrhizal citrus plants release a distinct profile of volatile organic compounds (VOCs) that function as airborne signals. These volatiles effectively "prime" the immunity of neighboring plants—both of the same and different species—thereby extending protection across the canopy. We analyzed the specific metabolite signals released by mycorrhizal citrus post-infestation and evaluated their impact on the immune readiness of neighboring vegetation. Our results suggest that AMF symbiosis serves as a critical catalyst for ecosystem-wide defense signaling. These insights highlight the potential of leveraging mycorrhizal associations and inter-plant communication as sustainable tools for integrated pest management in horticultural systems.

P.2.2. Inter-kingdom auxin signaling drives bacterial plant adaptation, plant growth promotion, and rhizosphere colonization. Miguel A. Matilla (EEZ-CSIC, Spain)

Inter-kingdom communication between plants and their associated microbiota is central to plant development and environmental adaptation. Indole-3-acetic acid (IAA) is the primary auxin in plants and regulates plant growth and development, while also functioning as a signal molecule that modulates bacterial physiology and behavior. Here, we show that IAA induces extensive transcriptomic reprogramming in the biocontrol rhizobacterium *Serratia plymuthica* A153. Exposure to IAA alters antibiotic production and enhances resistance to antibiotics and other toxic compounds by activating various adaptive responses. In addition, IAA promotes bacterial motility while strongly inhibiting biofilm formation through reductions in c-di-GMP levels. Although IAA produced by plant growth-promoting bacteria typically stimulates plant growth, excessive auxin levels can be detrimental to plant physiology, making auxin homeostasis essential. We demonstrate that the beneficial plant-associated bacterium *Pseudomonas putida* 1290, which can use IAA as a nutrient, exhibits chemotaxis toward IAA and plays a functional role in auxin turnover in planta. The IAA degradation pathway was found to be essential for reversing auxin-mediated growth inhibition in tomato and maize, both in vitro and under microcosm conditions. Moreover, bacterial IAA catabolism prevented the formation of tumor-like structures in maize roots caused by high auxin levels. Competitive colonization assays further revealed that IAA catabolism enhances bacterial fitness in the rhizosphere, particularly under high-auxin conditions. Collectively, our findings highlight the complexity of IAA-mediated signaling in beneficial plant-associated bacteria and identify bacterial IAA catabolism as a mechanism of metabolic signal interference that contributes to auxin homeostasis *in planta* and promotes successful rhizosphere colonization.

P.2.3. Nutrient transfer in *Colletotrichum tofieldiae*–plant interactions under P and N limitation. Gonzalo Monteoliva Garcia (Centro de Biotecnología y Genómica de Plantas, CBGP, Spain)

Authors: Gonzalo Monteoliva-Garcia, Johannes Gaertner, Marcel Bucher, Soledad Sacristán.

Fungal endophytes (FEs) are increasingly recognized for their ability to enhance plant performance in nutrient-poor environments. Establishing a fungus-plant stable mutualistic association requires reciprocal gains for both partners: plants would typically supply organic carbon (C) to the fungus, while receiving essential nutrients such as phosphorus (P) or nitrogen (N) in return. However, the molecular mechanisms governing this reciprocal nutrient exchange in FE remain poorly understood, despite the crucial importance of this knowledge for deciphering plant–fungus symbioses and for developing strategies to harness beneficial microbiota for sustainable crop production. The fungal root endophyte *Colletotrichum tofieldiae* (Ct) has been shown to promote growth in *Arabidopsis thaliana*, tomato (*Solanum lycopersicum*), and maize (*Zea mays*), although its plant colonization patterns and growth-promoting effects vary across host species and nutrient conditions, suggesting that nutrient exchange mechanisms differ among plant–fungus combinations. We combined physiological and transcriptomic approaches to dissect nutrient dynamics during Ct interactions with tomato. We demonstrate that Ct promotes plant growth under P or N deficiency both individually and in combination. Quantification of fungus to plant nutrient fluxes indicates that Ct transfers P to the plant in a N dependent manner. In parallel, we are analyzing RNA-seq data from both symbiotic partners under different nutritional regimes and comparing it with the interaction in Arabidopsis and maize. This approach reveals general and host specific transcriptional signatures associated with nutrient exchange and candidate plant and fungal transporters that may mediate bidirectional nutrient flow. This work has been funded by Severo Ochoa (SO) Program for Centers of Excellence CEX2020-000999-S (2022 to 2025) granted to CBGP and funded by MICIU/AEI /10.13039/501100011033, PRE2022-105238 funded by MCIN/AEI/10.13039/501100011033 and “ESF Investing in your future” to GM, and PID2021-123697OB-I00 and PID2024-161830OB-I00 funded by MCIN/AEI/10.13039/501100011033 and ERDF EU. JG´s work was conducted under Germany’s Excellence Strategy – EXC2048/1

P.2.4. Species-specific responses and the mechanism of tolerance of two *Rhizophagus* species to glyphosate. Christos Papadopoulos (University of Thessaly, Greece).

Pesticides are major environmental pollutants affecting humans, terrestrial and aquatic organisms, and beneficial soil microbes such as arbuscular mycorrhizal fungi (AMF). Glyphosate, the most widely used herbicide, targets the EPSPS enzyme in plants. While previous studies found that glyphosate affects AMF spore germination and sporulation-through direct toxicity or indirect effects via the host plant, its precise toxic mechanism remains elusive. We previously showed that *Rhizophagus irregularis* and *Rhizophagus clarus* responded differently to glyphosate across life-cycle stages. Following up, we performed complementary assays to explore the mechanism of toxicity of glyphosate on *R. irregularis*-sensitive and *R. clarus*-tolerant. Effects on root colonization were evaluated using the AMF-sandwich test. Changes in nuclear abundance in single spores were quantified via ddPCR targeting MAT-loci. Temporal development of the symbiotic mycelial network was monitored using high-throughput microscopic scanning. Glyphosate negatively affected R. irregularis at all stages, reducing spore nuclear abundance. In contrast, tolerant R. clarus, at low-concentrations, formed non-germinating spore-like structures, while high-concentrations increased nuclear abundance on spores. Still, glyphosate significantly altered the development and architecture of symbiotic mycelium in both species. EPSPS-mRNA was isolated, protein structures predicted with AlphaFold, and molecular docking indicated glyphosate targets AMF-EPSPS similarly to plants and non-major intraspecific differences were observed. These results suggest that the differing asymbiotic responses of the two AMF species to glyphosate may be spore-stage specific and linked to morphological differences, such as spore wall thickness. Ongoing experiments using 13C-labeled glyphosate on AMF spores traced by NanoSIMS aim to verify this hypothesis.

P.2.5. *Streptomyces hydrogenans* DEF17 as a dual-purpose seed bioinoculant enhancing iron nutrition and suppressing Fusarium wilt in tomato. Matias Pasquali (University of Milan, Italy)

Streptomyces hydrogenans DEF17 is a promising seed-applied bioinoculant that combines plant growth promotion, improved iron nutrition, and biocontrol-mediated disease suppression in tomato. DEF17 was originally selected for its siderophore production. It was taxonomically resolved as *S. hydrogenans* through hybrid genome assembly (Illumina + Nanopore) and its genetic potential was explored using dedicated bioinformatic pipelines. DEF17 seed treatment significantly reduced *Fusarium oxysporum f. sp. lycopersici* (Fol) wilt severity in planta, despite the absence of direct antagonism in dual culture assays, indicating a primarily plant-mediated mode of action. Root exudates of DEF17-treated tomato plants were more acidic and induced Fol phobotaxis. Metabolomics revealed a distinct exudate fingerprint enriched in specific compounds, consistent with rhizosphere reprogramming and modulation of defence-related metabolites. Under controlled greenhouse conditions, DEF17 seed treatment enhanced mineral uptake and plant performance. In particular, DEF17 markedly increased Fe(III) accumulation in tomato leaves under iron limiting conditions. Overall, DEF17 emerges as a mechanistically versatile Streptomyces strain that reshapes tomato root exudation and rhizosphere chemistry while simultaneously improving iron acquisition, supporting its development as a dual-purpose bioinoculant for integrated management of iron deficiency and Fusarium wilt in tomato.

P.2.6. The impact of *Trichoderma* elicitors in insect herbivores. Alegria Perez Guillen (EEZ-CSIC, Spain)

Insect pests represent a major threat to global agricultural yield. Conventional pesticides have long been used for pest control, but at a high environmental cost, highlighting the need for sustainable alternatives. Some beneficial microbes, such as *Trichoderma* species, enhance plant resistance to pests via Microbe-Induced plant Resistance (MIR). MIR relies on defence priming, allowing faster and/or stronger responses. However, the molecular mechanisms underlying MIR remain largely unknown, limiting its practical applications. Some secreted *Trichoderma* proteins (elicitors) modulate the plant jasmonic acid and ethylene pathways, also involved in MIR. Our hypothesis is that *Trichoderma* elicitors are sufficient to induce MIR. To test this hypothesis, four candidate elicitors from *Trichoderma harzianum* were heterologously expressed and applied to tomato seedlings for 24 h. To evaluate the priming effect, the elicitor-treated plants were challenged with the insect herbivores *Spodoptera exigua* and *Spodoptera littoralis* five weeks after the initial treatment. Noticeably, the elicitor-treated plants exhibited enhanced resistance, reflected by the reduced insect weight gain compared to controls. These findings show that individual Trichoderma elicitors can induce defence priming in tomato. The generation of *Trichoderma* mutant lines impaired in selected elicitors will allow us to determine whether these proteins are required for MIR onset. The identification of MIR eliciting proteins will allow their use as powerful tools in the investigation of MIR mechanisms and its ecological impact (MIR imprint).

P.2.7. Bioprospecting c-di-GMP activated exopolysaccharides in beneficial root-associated bacteria. Daniel Perez Mendoza (EEZ-CSIC, Spain)

The vast diversity of bacteria constitutes an essential resource for the discovery of novel exopolysaccharides (EPS) as raw materials with biotechnological applications. Beyond their technological relevance, EPS perform essential ecological functions in bacteria, including biofilm structuring, flocculation, root adhesion, desiccation tolerance, soil aggregation and signaling during plant interactions in both beneficial (e.g. Plant Growth Promoting Rhizobacteria-PGPR) and pathogenic contexts. These roles underscore the close relationship between EPS physicochemical properties and bacterial lifestyle. However, the identification of new EPS is often limited by their lack of production under laboratory culture conditions, as they are frequently cryptic and their biosynthesis is only activated in response to unknown environmental signals. The cyclic dinucleotide bis-(3'–5')-cyclic dimeric guanosine monophosphate (c-di-GMP) has emerged as a universal second messenger in bacteria and a common activator of many EPS, governing different plant-bacteria interactions. In this study, a genetic modification aimed at increasing intracellular c-di-GMP levels was combined with carbohydrate fingerprinting analysis to perform a high-throughput screening (HTS) of 330 environmental bacterial strains for the detection of c-di-GMP-activated EPS. Approximately 10% of the strains were identified as promising candidates for the c-di-GMP-dependent overproduction of novel EPS. Through this screening, we identified and characterized a new EPS in plant-interacting *Sphingomonas* strain, renamed SphC10. Under conditions of elevated c-di-GMP levels, this strain massively produces a labile membrane-associated EPS with an unusual monosaccharide composition compared to other biotechnologically relevant sphingans. These results position c-di-GMP as a versatile and attractive tool for exploring the hidden diversity of bacterial EPS with ecological value. *This study has recently been accepted for publication in Trends in Biotechnology.

P.2.8. Mechanism of growth promotion by endosymbiotic biofertilizers unraveled. Anna Maria Pirttilä (University of Oulu, Finland)

Bacteria and fungi that provide beneficial outcomes in crop plant growth and health, biofertilizers and biocontrol agents, are available on the market as products for agriculture. The strains are mainly based on plant-growth-promoting rhizobacteria (PGPR), as well as soil, epiphytic, and mycorrhizal fungi. However, there are challenges to their production and use, specifically the inconsistency of field performance. We have discovered endosymbiotic bacteria from plant shoot meristems that could be used as a component in synthetic communities. The endosymbionts have specific mechanisms of action, intrinsically manipulating the host functions for enhanced growth and stress tolerance. We performed comparative genome analysis on the endosymbiotic strains *Methylorubrum extorquens* DSM13060 and *Pseudomonas synxantha* DSM13080. Our results identified genes for versatile secretion systems and polyhydroxybutyrate synthesis, shared by both strains. Specifically, *P. synxantha* DSM13080 possesses a set of proteins with eukaryote-like domains, of which ankyrin repeat proteins, protein kinases, and leucine-rich repeat proteins, shared with *M. extorquens* DSM13060, could be involved in intracellular colonization and mediation of plant responses. The identified traits are distinct from previously reported plant-growth promoting strains. The endosymbionts therefore likely represent microbiome members valuable for biofertilizer or biocontrol preparations.

P.2.9. From roots to shoots: A HD-ZIP IV module linking arbuscular mycorrhizal symbiosis to systemic resilience. Beatriz Romero-Rodriguez (EEZ-CSIC, Spain)

Arbuscular mycorrhizal fungi (AMF) symbiosis can reshape plant physiology beyond nutrient acquisition, influencing tolerance to environmental stress and interactions with herbivores. While AMF are known to enhance plant resistance to herbivory, the regulatory mechanisms underlying this effect remain largely unexplored. Using RNA-seq, we identified a tomato HD-ZIP transcription factor induced in leaves of mycorrhizal plants following herbivory. To evaluate its function, we performed virus-induced gene silencing in tomato and transient overexpression in Nicotiana benthamiana. Overexpression increased larval growth of the leaf herbivores *Spodoptera littoralis* and *Manduca sexta*, whereas silencing reduced herbivore performance, indicating that this factor modulates plant-herbivore interactions. Notably, classical pathways regulating mycorrhiza-induced resistance were largely unaffected, suggesting that the effect occurs through non-canonical defense mechanisms. Metabolic profiling revealed reduced proline levels in silenced plants, which also exhibited impaired tolerance to desiccation stress, highlighting a role in osmotic adjustment. I*n silico* promoter analysis and microscopy suggested downstream targets involved in water stress responses and cuticle development, consistent with known HD-ZIP roles in epidermal differentiation. Together, these findings indicate that this transcription factor acts as a regulatory hub linking AMF symbiosis to herbivore tolerance through physiological and structural adjustments. By connecting belowground symbiotic signals to aboveground ecological outcomes, this study illustrates how mycorrhizal associations can enhance plant resilience and shape trophic interactions in both natural and managed ecosystems. Funding information: ERC-2023-COG: 101124883 MIMIR by European Union; PID2021-128318OA-I00 and PID2024-162058OB by MCIN/AEI/10.13039/ 501100011033 and 'ERDF A way of making Europe'.

P.2.10. Assessing bacterial fitness using a genome-wide approach. Marta Torres Bejar (University of Granada, Spain)

The diverse community of soil microorganisms inhabiting the rhizosphere plays an important role in plant nutrition and protection against pathogens. Consequently, promoting plant growth by harnessing the soil microbiome stands as a promising and sustainable alternative to agrochemicals. In order to increase crop yield and quality, numerous efforts have aimed at better understanding how plant colonization by soil bacteria is shaped. One needed step is having a genome-wide map of fitness determinants involved in plant colonization by a given bacterium, which offers a starting point for future actions such as the targeted improvement of plant colonization capabilities of beneficial bacteria, or the development of protection strategies against plant pathogens. Despite the great advances in our understanding of bacterial plant colonization, the extent to which factors such as plant growth substrate, abiotic and biotic stresses influence the fitness molecular mechanisms enabling bacteria to efficiently colonize plants remains poorly understood. To evaluate how genes that are important for plant colonization are influenced by such factors we use a genome-wide approach called randomly barcoded transposon mutagenesis sequencing (RB-TnSeq) in species from the Pseudomonadota and Bacteroidota phyla. Altogether, we demonstrate that RB-TnSeq can robustly define subtle colonization differences across conditions and that bacterial fitness for plant colonization is strongly influenced by different factors, notably plant growth substrate. Globally, our data shows the importance of considering how different parameters impact bacterial fitness in plant colonization studies. This is especially relevant when the aim is to identify fitness genes to design more effective rhizosphere management strategies or engineer strains to improve their plant colonization efficiency

P.2.11. Symbioprotein Ferritin - Evidence for a dual role of Ferritin(s) in legume-*Rhizobium* symbiosis. Stefanie Wienkoop (University of Vienna, Austria)

Ferritins are a universal family of iron-storage proteins that play a critical role in iron homeostasis across all domains of life. Their evolutionary history also highlights their important role in the interaction between different organisms. In Legume-*Rhizobium* symbiosis, ferritins have been shown to play a crucial role in regulation of iron homeostasis and protection against oxidative damage in nitrogen fixing nodules. While much is known about their structure and function, we want to demonstrate the reciprocal regulation of the distinct plant ferritins when symbiotically interacting with rhizobia. We therefore compared *Rhizobium* symbiotic with non-symbiotic *Medicago truncatula* plants. We investigated stoichiometry and N2-fixation rates of three ferritin paralogs by using knockdown mutants This enabled to tackle the major ferritins to specify their roles during symbiotic N2-fixation and during induced systemic stress response. The results strengthen the function of ferritins in symbiosis evolution and crosstalk between plant biotic- and abiotic stress regulation.

P.2.12. Evaluating the impact of nitrate on nodulation in wild and cultivated chickpea genotypes. Melike Bakir (Erciyes University, Turkey)

Symbiotic nitrogen fixation (SNF) in chickpea (*Cicer arietinum* L.) plays a vital role in sustainable agriculture by reducing dependence on synthetic nitrogen fertilizers. This process relies on the establishment of effective nodules through interaction with *Mesorhizobium ciceri*. However, elevated soil nitrate levels can suppress nodulation and limit the efficiency of SNF. While nitrate inhibition has been extensively studied in cultivated chickpea, the response of wild *Cicer* species under nitrate stress remains largely unexplored. This study aims to evaluate the effects of nitrate on nodulation in selected wild and cultivated chickpea genotypes. Plants inoculated with *M. ciceri* were grown under controlled conditions and subjected to varying nitrate concentrations. Nodulation traits, including nodule number, size, biomass, and distribution, were recorded to assess genotypic variation in response to nitrate application. By comparing wild and cultivated genotypes, this study seeks to identify chickpea lines that maintain nodulation under nitrate stress. The findings will contribute to a better understanding of nitrate tolerance in *Cicer* species and support the development of chickpea varieties with improved nitrogen-use efficiency.

P.2.13. Some insight into the structure-function relationship of plant hemoglobins. Manuel Becana (EEAD-CSIC, Spain)

Plants contain symbiotic hemoglobins, which are present only in the nodules of legumes (leghemoglobins; Lbs), actinorhizal plants and *Parasponia*, and nonsymbiotic hemoglobins (Glbs), which occur in most, if not all, plant organs. Typically, Glbs are categorized into three classes based on phylogeny and biochemical properties. However, only a few functions have been assigned to plant hemoglobins. Thus, Lbs carry oxygen to the bacterial partner inside the nodules whereas class 1 Glbs are involved in the homeostasis of nitric oxide, a key signal molecule. Class 2 Glbs share sequence homology with Lbs and class 3 Glbs share sequence homology with bacterial truncated hemoglobins, but in both cases their functions are uncertain. Several difficulties encountered in determining hemoglobin functions are the scant number of available crystal structures, the few data on the affinities of the proteins for potential heme ligands, and the relatively small number of hemoglobin mutants that have been phenotyped under physiological and stress conditions. Here, we will show the crystal structure of Glb2-1, as well as metabolomic and proteomic data of glb1-1 and glb2-1 mutants of *Lotus japonicus*. These data indicate that the two hemoglobins perform non-overlapping functions. In particular, we have identified amino acid residues (Tyr31, distal His 64, Cys65) of Glb2-1 that are critical for the stability of bound oxygen. Also, we have found changes in metabolites and proteins related to the defense response that differ in the two mutants, and which include salicylic, jasmonic, and pipecolic acids, as well as the class-10 pathogenesis related protein 1.

P.2.14. Exploring half-sized ABCG transporters in *Medicago truncatula* root nodules. Joanna Banasiak (Polish Academy of Sciences, Poland)

Authors: Joanna Banasiak, Abinaya Manoharan Geetha Ponmalar, Michał Jasiński.

Legume species from the nitrogen-fixing root nodule clade have evolved the ability to establish symbiosis with rhizobial bacteria, enabling biological nitrogen fixation and growth in nitrogen-poor soils. Root nodules are central to this interaction, serving as specialized organs for bacterial accommodation, nitrogen fixation, and nutrient exchange. While the mechanisms of nutrient transfer between symbionts are well understood, the roles of transporters in nodule development, maintenance, and overall function remain largely unexplored. ATP-Binding Cassette (ABC) transporters of the G subfamily are promising candidates for supporting proper nodule formation and activity, through phytohormone translocation, establishment of oxygen diffusion barriers, or modulation of Reactive Oxygen Species (ROS) accumulation. Based on phylogenetic analysis, we selected half-sized ABCG transporters from Medicago truncatula belonging to three subclades: i) suberin-, ii) hormone-, and iii) ROS-related. Using SYMbiMICS RNA-seq data from roots and laser-dissected nodule zones, we assessed their expression patterns. In each subclade, a single transporter showed high or nodule-specific expression, highlighting its potential role in nodule function and nodule-driven specialization. Future functional characterization, combining genetic, molecular, and physiological approaches, will be crucial to elucidate the specific roles of these transporters in nodules.

P.2.15. Role of RelA in early and late stages of the *Sinorhizobium meliloti–Medicago sativa* symbiosis. Cristina Carvia Hermoso (EEZ-CSIC, Spain)

The mutualistic association between the soil bacterium *Sinorhizobium meliloti* (Sm) and alfalfa (*Medicago sativa*) results in the formation of nitrogen fixing root nodules. This developmental program depends on a tightly coordinated molecular exchange between both partners, as well as on the bacterium's capacity to withstand multiple stresses encountered in the rhizosphere and within plant tissues. The stringent response (SR) is a global regulatory pathway that enables bacteria to adapt to adverse conditions and is governed by the alarmone nucleotides (p)ppGpp, whose intracellular levels are controlled by members of the RSH (RelA/SpoT Homologue) protein family. In a forward genetic screen aimed at identifying determinants of surface motility in the alfalfa symbiont Sm GR4, a *relA*::Tn5 mutant was recovered. Notably, unlike the nodulation defective phenotype described for *relA* mutants derived from strain Rm1021, disruption of *relA* in the GR4 background does not abolish nodule formation on alfalfa. This finding points to functional divergence of the SR regulatory network among Sm strains. Phenotypic characterization of GR4 *relA* mutants indicates that the SR contributes to multiple stages of the symbiotic process, from early root colonization to later nodule cell infection, influencing essential traits such as competitiveness and nitrogen fixation efficiency. Ongoing transcriptomic and functional analyses aim to unravel the molecular mechanisms underlying the compromised symbiotic performance observed in GR4 *relA* mutants.

P.2.16. A beneficial bacterial association regulates metabolic responses and flowering time in *Arabidopsis thaliana* under nitrogen deficiency. Berengere Decouard (Institute of Plant Sciences Paris Saclay, IPS2, France)

Authors: Bérengère Decouard, Caroline Mauve, Amina Ilyas, Axel de Zélicourt.

Nitrogen fertilization sustains crop productivity but depends on energy-intensive industrial processes and can contribute to environmental degradation. Beneficial plant-associated microbes represent a promising approach to improve plant performance under reduced nitrogen input. While nitrogen-fixing symbioses have been well studied, the influence of non-nitrogen-fixing beneficial bacteria on plant developmental responses to nitrogen availability remains less understood. Previous studies showed that SA187 enhances plant growth specifically under nitrogen limitation (1 mM nitrate), with an increase in fresh weight of over 100%, associated with enhanced nitrogen and nitrate content. This growth promotion depends on ethylene signalling and high-affinity nitrate transporters (HATS), as the *ein2*, *nrt2.5*, and *nrt2.6* mutants do not exhibit enhanced growth. To gain further insight into the influence of SA187 on plant metabolism, transcriptomic and primary metabolomic datasets (GC-MS profiling and HPLC amino acid measurements) were integrated using DIABLO multiblock analysis. This analysis revealed that bacterial inoculation across nitrogen regimes causes coordinated variation between primary metabolites and genes involved in flowering-time regulation, prompting further investigation of reproductive development. Interestingly, SA187-inoculated plants flowered 4 days earlier under nitrogen limitation. Inoculated plants also produced more siliques, indicating that accelerated flowering occurred alongside maintained reproductive performance. Importantly, this developmental shift was also observed in the ein2 mutant, suggesting that flowering modulation operates independently of ethylene signalling and is mechanistically distinct from the previously described growth promotion during early vegetative stages. Together, these results show that SA187 association alters plant developmental responses to nitrogen limitation and links metabolic regulation with changes in flowering time.

P.2.17. m6A RNA methylation as a regulatory layer in plant responses to microbial volatile compounds. Rafael Jorge Leon Morcillo (IHSM-CSIC, Spain)

Microbial volatile compounds (VCs) trigger profound changes in plant growth, photosynthesis, and stress tolerance through multilayered regulatory networks integrating retrograde signalling, metabolic reprogramming, and long-distance root–shoot communication. While transcriptional responses to microbial VCs are well documented, the contribution of post-transcriptional regulation remains largely unknown. We hypothesized that N6-methyladenosine (m6A) RNA methylation acts as a regulatory hub connecting microbial volatile perception with downstream gene expression control. To test this idea, we compared transcriptomic responses of Arabidopsis plants exposed to fungal VCs with datasets from mutants impaired in m6A methylation. A substantial overlap was identified between VC-regulated genes and those misregulated in the *mta* mutant, indicating that reduced m6A activity partially mimics VC-induced transcriptional reprogramming. Consistently, several Arabidopsis mutants impaired in genes encoding components of the m6A methylation complex exhibited attenuated responses to microbial VCs, while transcripts encoding these regulatory factors were themselves differentially regulated upon VC exposure. Functional enrichment analyses linked shared gene sets to photosynthetic regulation, primary metabolism, and stress-associated pathways. Co-expression and transcript feature analyses further suggest that m6A-dependent regulation may influence mRNA stability and translation efficiency of VC-responsive genes, potentially mediated by specific reader proteins. These findings support a model in which microbial VCs reshape plant physiology through epitranscriptomic control of gene expression. Our work positions m6A methylation as a key mechanistic layer in plant–microbe communication and highlights epitranscriptomic regulation as a promising target to enhance plant performance and stress resilience.

P.2.18. Unraveling the role of SnRK1 in the regulation of nodulation in soybean. Joren Desloovere (VIB-PSB, University of Ghent, Belgium)

Legumes such as soybean acquire nitrogen through an endosymbiotic interaction with nitrogen-fixing bacteria called rhizobia. These bacteria colonize organ-like root structures known as nodules. They convert atmospheric nitrogen into plant-available forms such as ammonia through biological nitrogen fixation. Because this process is highly energy-intensive, rhizobia rely on carbon sources supplied by the plant, primarily malate and succinate derived from sucrose. Efficient nodulation and nitrogen fixation therefore depend on a tightly regulated carbon/nitrogen (C/N) balance linked to photosynthetic activity and N availability. A key integrator of plant C and N status is the energy-sensing kinase SUCROSE NON-FERMENTING1-RELATED KINASE1 (SnRK1). Its catalytic subunit, KIN10, phosphorylates metabolic enzymes and transcription factors to regulate growth in response to metabolic changes and stress. While SnRK1 plays an important role during plant development, its function in nodulation remains largely unknown. Interestingly, we recently identified a KIN10 homolog, KIN10b, that is specifically expressed in infected nodule cells. This was revealed through qRT-PCR, our in-house single-nucleus RNA sequencing datasets covering immature, mature, and senescing nodules, and GUS reporter analyses. These observations suggest a specialized role for SnRK1 signalling in the regulation of nodulation. Using a cutting-edge SnRK1 activity biosensor, we aim to characterize the spatial and temporal dynamics of SnRK1 during nodule development and after nodulation perturbations including shoot decapitation and the addition of nitrate. Combined with interactomics and snRNA-seq, this approach will enable us to identify cell-type-specific SnRK1 regulatory networks involved in the regulation of nodulation and C/N signalling.

P.2.19. When help turns costly: arbuscular mycorrhizal effects on legume physiology under contrasting drought intensities. Karolina Jarzyniak (Poznan University of Life Sciences, Poland)

Drought is one of the most severe constraints on plant productivity, and its increasing frequency under climate change highlights the need for strategies that enhance crop resilience. Arbuscular mycorrhizal fungi (AMF) are widely recognized as beneficial soil microorganisms that improve plant access to water and nutrients, and may mitigate the negative effects of water deficit. However, the magnitude of these benefits varies with plant species, nutrient status, and drought severity. This study examined how the arbuscular mycorrhizal fungus *Rhizophagus irregularis* shapes the response of the model legume *Lotus japonicus* to short- and long-term drought by comparing AM-inoculated plants grown under reduced phosphorus supply with non-mycorrhizal controls cultivated either at the same low phosphorus level or under phosphorus-sufficient conditions. This design enabled us to distinguish the effects of symbiosis from those resulting solely from improved nutrition. A comprehensive set of morphological and physiological traits was assessed, including biomass allocation, root system architecture, relative water content, proline accumulation, and photosystem II efficiency. AMF markedly improved plant performance under mild drought to a degree comparable to or exceeding that achieved through phosphorus supplementation. In contrast, under prolonged and severe drought, the benefits of symbiosis diminished and could even become detrimental, indicating a shift in the interaction's cost–benefit balance. These findings highlight the context-dependent nature of AMF-mediated drought tolerance and emphasize the importance of integrating nutrient status and stress intensity when evaluating microbial contributions to plant resilience. Such insights are essential for optimizing the use of beneficial soil microorganisms in sustainable agriculture.

P.2.20. Unraveling the secrets of small RNAs in arbuscule-containing cells. Luisa Lanfranco (University of Turin, Italy)

Roots colonized by arbuscular mycorrhizal (AM) fungi are a highly heterogeneous cellular environment that includes different plant cell types establishing interactions with different fungal structures. Analyses performed on whole roots can thus underestimate the cellular and molecular complexity of this plant-fungus association. In this work the laser microdissection technique was used to uncover the gene regulatory networks associated to arbusculated cells with a specific focus on small RNAs (sRNAs), which are emerging as novel regulators of the AM symbiosis. RNA-seq was performed on laser microdissected-isolated cell populations to profile the sRNA from arbusculated cells (ARB) and cortical cells from non mycorrhizal root (CON) as a control. *M. truncatula* mapping sRNAs are mainly 21 nt long with uracil at the 5' end in all samples while *R. irregularis* mapping sRNAs tend to be 22 nt long, mainly with adenine at the 5' end. Gene ontology analysis for biological processes on plant putative targets of Medicago miRNAs showing up-regulation in ARB vs CON highlighted categories related to root morphogenetic processes and lateral root development, lignin catabolic processes and defence response. This dataset is a powerful tool to identify sRNA-mediated gene regulatory mechanisms active in arbusculated cells and can be exploited to study plant endogenous gene regulation by miRNA as well as fungus to plant or plant to fungus events of cross-kingdom RNA interference.

P.2.21. Molecular dialogues inside the root: Priming plants to withstand drought tolerance. Kalliopi Papadopoulou (University of Thessaly, Greece)

Authors: Maria Feka, Olga Tsiouri, María Manresa-Grao, Loukia M. Kellari, Sotirios Vasileiadis, Victor Flors, Kalliope K. Papadopoulou

Drought stress is a major limitation to crop productivity, affecting plant growth, physiology and yield. Beneficial endophytic fungi have emerged as promising tools for enhancing plant tolerance to abiotic stresses through modulation of host signaling and metabolism. *Fusarium solani* strain K (FsK), a soil-borne endophyte isolated from tomato roots, has previously demonstrated protective effects against biotic stressors; however, its role in drought tolerance remains less explored. In this study, we assessed the ability of FsK to enhance drought resilience in tomato plants under reduced irrigation conditions. FsK-inoculated and non-inoculated plants were subjected to water deficit, followed by physiological, transcriptomic, and metabolomic analyses. FsK-colonized plants exhibited significantly improved performance, including higher relative water content and increased biomass accumulation compared to controls. Transcriptomic analysis revealed the upregulation of key drought-responsive pathways, particularly those associated with abscisic acid (ABA) and jasmonic acid (JA) signaling. Additionally, genes encoding WRKY transcription factors and components of mitogen-activated protein kinase (MAPK) cascades were induced, indicating activation of complex regulatory networks involved in stress adaptation. Metabolomic profiling showed enhanced accumulation of phenolic compounds and organic acids, including malic acid, suggesting improved antioxidant capacity and osmotic adjustment. Our findings indicate that FsK functions as a priming agent, enhancing tomato tolerance to drought stress through coordinated regulation of gene expression and metabolite profiles. This study highlights the potential application of beneficial endophytes such as FsK in sustainable strategies to improve crop resilience under water-limited conditions.

P.2.22. SynComs affected sorghum-associated fungal communities in varied plant compartments. Stefan Shiilev (Agricultural University – Plovdiv, Bulgaria)

Sorghum (*Sorghum bicolor*) is a key cereal crop in semi-arid agriculture, yet its productivity is often compromised by soil-borne fungal pathogens. This study evaluated the effects of Syncoms bioinoculants on the microbial community structure and ecological function across diverse plant compartments – soil, root, seed, and stem, using high-throughput ITS amplicon sequencing and advanced statistical analyses. Alpha and beta diversity metrics showed that bioinoculant treatment significantly reduced overall richness and evenness, particularly in stem and seed tissues, while promoting the dominance of specific beneficial taxa. PERMANOVA confirmed significant differences in community composition between treated and control groups (p<0.01), with the greatest shifts observed in aboveground plant parts. LEfSe analysis identified *Trichoderma* and *Mortierella* as biomarkers of treated samples, whereas *Fusarium*, *Alternaria*, and *Penicillium* were more abundant in controls. Functional annotation using FUNGuild revealed that the enriched taxa in treated samples were predominantly saprotrophs or symbiotrophs, whereas the control samples were characterised by a higher proportion of pathogenic fungi. These results suggest that bioinoculant application selectively enriches fungal taxa with ecological roles associated with promoting plant health, suppressing pathogens, and facilitating nutrient cycling. By restructuring the sorghum-associated microbiome toward beneficial functional groups, SynComs inoculants offer a promising strategy for sustainable disease management and agroecological improvement. The study highlights the utility of microbial inoculants in shaping rhizosphere and endosphere communities to support crop resilience and productivity.

P.2.23. Beyond rhizobia: Exploring the dynamic interplay between nodulation-enhancing endophytes and legume physiology. Jeanne Veliscek (Institute of Plant Sciences Paris-Saclay, IPS2, France)

Authors: Jeanne Veliscek, Florian Frugier, Axel de Zélicourt

Nitrogen is a fundamental nutrient for plant growth, development, and productivity. Legumes play an essential role in sustainable low-input agriculture due to their unique capacity to establish symbiotic interactions with nitrogen-fixing rhizobia. Beyond rhizobia, a diverse range of endophytic bacteria can colonize plant tissues and exert beneficial effects, including the enhancement of symbiotic nodulation in legumes. In many cases however, mechanisms associated to their endophytic colonization and to their impact on plant growth remain poorly understood. We thus initiated a project aiming to (1) elucidate the role of nodulation-enhancing endophytes using an integrative approach in the model legume *Medicago truncatula*, (2) identify the molecular pathways underlying their colonization efficiency, notably depending on nitrogen availability. Preliminary results led to the identification of several bacterial candidates able to significantly enhance plant growth and nodulation in *M. truncatula*. Ongoing investigations focus on determining their spatial localization within plant root and nodule organs, defining the nutrient conditions that modulate their activity, notably in relation to nitrogen, and assessing whether their effects are local and/or systemic.

P.2.24. Molecular mechanisms of biofilm formation and salt adaptation in the plant-beneficial strain *Stutzerimonas stutzeri* MJL19. Manuel Espinosa-Urgel (EEZ-CSIC, Spain)

Stutzerimonas stutzeri MJL19 was isolated from the rhizosphere of plants growing in a salt flat in Argentina and has been shown to efficiently colonize the seeds and roots of soybean (*Glycine max* L.), stimulating germination and plant growth, particularly under saline stress. We have begun to explore the genetic determinants involved in extracellular matrix production and biofilm formation by *S. stutzeri* MJL19, their influence on plant physiology, and their connection with the adaptation to salt stress. Cellulose and the species-specific exopolysaccharide Sea, are important structural elements with different roles, the second being key for the protective effect of MJL19 against salt stress in soybean plants. Evidence also indicates the participation of functional amyloids in the competitive fitness of the bacterium in the rhizosphere. In addition, the role of some regulatory and signal transduction elements has been analyzed, particularly in relation to the intracellular second messenger cyclic diguanylate. Our results highlight the multiple factors that influence adaptation of *S. stutzeri* to the rhizosphere environment.

WG3 session

P.3.1. Variable symbiotic capacity of pea varieties - a factor to consider in sustainable agroecosystems?

Martina Janouskova (Institute of Botany-Czech Academy of Sciences, IBOT-CAS, Czech Republic)

Crop varieties can differ in their symbiotic capacity, i.e. the ability to support root-associated symbionts and profit from them. Thus, abundance of microbial symbionts in arable soil and their contribution to a variety of ecosystem services, may depend not only on the crop species planted, but also on the variety used. We address the importance of crop variety for the development of root-associated symbionts and maintenance of their diversity in arable soils using pea (*Pisum sativum*), an important soil-improving crop increasingly utilized in crop rotations and intercropping systems. In a first step, we performed a screening of 15 pea varieties in three soil-nutritional conditions. Based on this, we determined varieties supporting higher or lower root colonization by rhizobia and arbuscular mycorrhizal fungi (AMF). Root colonization by AMF was correlated with mycorrhizal responses and nodule weight, but unrelated to root traits. In a next step, the varieties are being screened in field conditions to determine whether they display consistent differences in root colonization and variability in the symbiont community composition. Altogether, our research will provide a realistic assessment on whether variable symbiotic capacity of pea varieties is a factor to consider when incorporating pea into sustainable cropping systems.

P.3.2. Legacy effect of short-term biocide exposure on soil microbial communities with lasting functional consequences.

Caroline Krug Vieira (Czech Academy of Sciences, Czech Republic)

Soil microbial diversity sustains key ecosystem functions, yet it remains unclear whether microbial communities exposed briefly to various kinds of biocides lead to persistent functional shifts. To address this, we conducted a two-stage experiment using eight biocides (four antibiotics and four fungicides), applied individually, to suppress specific microbial guilds in soil microcosms. In the first stage of the experiment, a single brief biocide pulse was imposed, and microbial population size, community composition, and metabolic activity were assessed via qPCR, DNA sequencing and respiration of ^{13}C-glucose after several weeks of incubation. Antibiotic treatments reduced ^{13}C-glucose respiration, whereas among fungicides, only myclobutanil increased respiration relative to controls. Biocide identity produced compound-specific effects on bacterial and protist communities, while fungal responses were variable. We observed declines in plant-beneficial taxa (e.g., *Rhizobium* and *Azospirillum*) alongside enrichment of stress-tolerant taxa, such as *Altererythrobacter*, *Ammoniphilus*, *Streptomyces*, *Cladosporium*, *Penicillium*, *Colpoda* and *amoebae*. In the second stage, pre-treated communities were introduced into a plant bioassay to assess growth of mycorrhized and non-mycorrhized *Cichorium intybus* and associated soil processes. Microbial shifts persisted for up to 47 days after transfer to the plant–soil system. Mycorrhiza enhanced soil carbon stabilization, and soils treated with myclobutanil showed higher ^{13}C retention than antibiotic-treated soils. Chitinase and acid phosphatase activities were greater in mycorrhizal treatments. Overall, brief biocide exposure generated persistent microbial legacy effects that affected arbuscular mycorrhizal colonization, plant biomass, enzyme activities, carbon stabilization and nitrogen uptake, with clear consequences for soil functioning.

P.3.3. ***Lupinus albus*** **as an alternative source of vegetable protein based on its ability to fix nitrogen.** Miguel Lopez Gomez (University of Granada, Spain)

There are three species of lupin (*L. albus*, *L. angustifolius*, *L. luteus*) of great agronomic interest, although they differ in their soil and climate requirements. *L. albus* has a high nitrogen fixation capacity, making it a good candidate for low-input agriculture and an alternative to soybean as a source of vegetable protein. The objective of this study is to identify and select cultivars adapted to our soil and climate conditions for the use of lupin as an emerging source of vegetal protein. To this end, yield, drought resistance and tolerance to the presence of pathogens in the soil, as well as nitrogen-fixing capacity, have been taken into account. Significant differences have been observed between different species in terms of their sensitivity to the presence of pathogens in the soil, with *L. albus* (cv. Celina) showing greater sensitivity with clear symptoms of rot in non-sterile soils. In terms of nitrogen fixing capacity, nodular biomass was almost double in *L. albus* compared to *L. luteus* and *L. angustifolius*. Data concerning the response to drought conditions among different cultivars and species will be also presented in this work.

P.3.4. MICROBES-4-CLIMATE Project: Understanding microbiome-plant-soil-environment interactions for climate-resilient ecosystems. Malek Marian (AIT Austrian Institute of Technology GmbH, Austria)

Terrestrial biodiversity and ecosystems are being challenged by climate change and environmental degradation with threats to agricultural and forestry ecosystems. Microorganisms constitute the life support system of the biosphere. However, the impacts of climate change-induced stresses on the microbiome assembly, structure, and function, as well as the microbiome contribution to crop stress mitigation and adaptation remain poorly understood. MICROBES-4-CLIMATE provides the global research community (users) with coordinated access to a cluster of complementary, world-class Research Infrastructures offering integrated advanced services, training, and scientific and technical support. Through its excellence-driven Transnational Access program, the project enables users to conduct curiosity-driven research addressing terrestrial biodiversity and ecosystems, and the complex microbiome-plant-soil-environment interactions underlying climate resilience and mitigation. By fostering high-quality, interdisciplinary research, the initiative advances knowledge on these interactions and supports the development of climate-resilient ecosystems. Within this framework, Work Package 3 focuses on the establishment and validation of synthetic microbial communities (SynComs) to enhance drought resilience in agricultural systems. Wheat plants and associated soils were collected from drought-affected regions in Greece, Italy, and France, and their microbiome are being characterized using both culture-dependent and culture-independent approaches. These data will guide the rational design of SynComs aimed at improving wheat drought tolerance under field-relevant conditions. Acknowledgments: Work supported by the awarded Horizon Europe MICROBES-4-CLIMATE Project (Microbial services addressing climate change risks for biodiversity and for agricultural and forestry ecosystems: enabling curiosity-driven research and advancing frontier knowledge; Grant agreement ID: 101131818).

P.3.5. Host-plant-derived rhizospheric consortia selectively enrich beneficial symbionts and restore soil functions in degraded quarry ecosystems. Georgios Leventis (Agricultural University of Athens, Greece)

Quarrying operations cause severe environmental degradation, yet soil microbial communities and plant-microbe interactions remain underutilized in restoration practice. Root-associated microbial communities are pivotal for plant performance, stress tolerance and nutrient cycling, but their application as inoculation tools, particularly in fragile, water-limited Mediterranean ecosystems based on indigenous flora remains underexplored. We investigated how indigenous whole rhizospheric bio-communities derived from conspecifics (Own-Crude) or a pioneer grass species (*Hyparrhenia hirta*; Crude-HH) reshape prokaryotic and fungal communities in the rhizosphere and endoroot compartments of two native legume species, and how these shifts translate into plant performance and soil functional outcomes. Inoculum source drove distinct and compartment-specific microbiome restructuring. Own-Crude significantly shifted endoroot prokaryotic communities, selectively enriching rhizobial-annotated ASVs (Rhizobiaceae), which co-occurred with increased nodulation and AMF colonization — dominated by *Rhizophagus*, *Dominikia* and *Septoglomus* — and coincided with enhanced plant growth, nutrient status and N- and P-cycling enzyme activities. Crude-HH reshaped both rhizospheric and endoroot prokaryotic communities through selective enrichment of

Streptomycetaceae ASVs, and induced AMF taxon-specific shifts; however, these community changes did not translate into improved symbiosis or plant performance under present conditions, although they may prove advantageous under stress. Both inoculation treatments converged prokaryotic and fungal assemblages toward more homogeneous community structures relative to uninoculated controls. These findings demonstrate that inoculum source determines the functional relevance of microbiome shifts, with host-plant-derived communities establishing ecologically meaningful plant-microbe interactions. We propose that this microbiome-based approach may be further developed and serve as a cost-effective strategy for enhancing sustainable revegetation and soil restoration in barren quarry areas.

P.3.6. Revalorization of distillation residues of medicinal plants as soil amendments: impacts on soil health and microbiota. Francesco Berruto (University of Turin, Italy)

The increasing interest in sustainable agriculture has been driven by growing awareness of the environmental impacts of conventional farming practices. Adopting technological innovations and circular economy practices in waste management can significantly enhance efficiency and reduce ecological footprint of practices such as improper disposal. Medicinal and aromatic plants (MAPs) are rich in essential oils and usually undergo distillation processes, generating residual biomasses that should be considered valuable resources since they are still rich in bioactive compounds. This work aims to explore the valorization of these residues as soil amendments and to evaluate their effects on soil health and crops and the impacts on the assembly of microbial communities. Two experiments have been planned. A two-years open-field trial has been carried out, adding characterized MAP residues as soil amendments in a maize field in both years. Soil and root samples, delivered twice a year, have undergone DNA extraction, sequencing and metabarcoding analysis for characterizing fungal, prokaryotic and arbuscular mycorrhizal fungi (AMF) communities. Prokaryotic communities show a clearer differentiation among treatments than fungi, suggesting greater sensitivity to soil management. Results indicate that multi-year management is needed for community restructuring. A greenhouse experiment is ongoing for evaluating the effects of partial replacement of peat with mint distillation residues, combined with AMF inoculation, on growth and quality of MAP species. Physical-chemical analyses of soils have been performed in both experiments, showing significant differences among different conditions.

WG4 session

P.4.1. Enhancement of vegetative establishment during acclimatization and tuber production in micropropagated potato by individual and consortium Plant Growth-Promoting Bacteria. El Hadi Erbiai (Universidade de Trás-os-Montes e Alto Douro, UTAD, Portugal)

Potato (*Solanum tuberosum* L.) micropropagation ensures the production of disease-free planting material; however, plantlet establishment during acclimatization is often constrained by physiological stress, leading to reduced survival and yield, and frequently requires intensive chemical inputs. Plant growth-promoting bacteria (PGPB) offer a sustainable alternative to improve plant establishment and yield through their functional traits. In this study, three native PGPB isolated from vineyard samples in the Douro Wine Region, *Bacillus*, *Burkholderia*, and *Streptomyces*, were evaluated as individual and consortium inoculants for micropropagated potato under greenhouse acclimatization. The isolates showed strong antagonistic activity against major fungal pathogens (*Fusarium oxysporum*, *F. solani*, *Botrytis cinerea*, and *Alternaria alternata*) and exhibited key beneficial traits, including indole-3-acetic acid production, siderophore synthesis, and phosphate solubilization. Eight treatments (three single strains, three two-strain mixtures, one three-strain consortium, and a non-inoculated control), each with six replicates, were applied at transplanting. All bacterial treatments improved vegetative establishment compared with the control, increasing plant height, shoot, and root biomass. The *Burkholderia* + *Streptomyces* consortium produced the tallest plants and greatest shoot biomass, while *Bacillus* significantly stimulated root development. In addition, *Burkholderia* alone increased tuber number, and the three-strain consortium resulted in the highest total tuber weight per plantlet. These results indicate that native PGPB, particularly when combined in compatible consortia, can enhance both acclimatization efficiency and tuber production of micropropagated potato. Overall, this study illustrates how beneficial bacteria from one perennial crop system can be repurposed as bioinoculants for another, contributing to more sustainable and resilient crop production.

P.4.2. Community composition and network architecture of arbuscular mycorrhizal fungi in a tropical forest. Damilola Olanipon (CNRS, Montpellier, France)

Tropical rainforest productivity relies on efficient nutrient cycling, largely facilitated by symbiotic associations between plants and arbuscular mycorrhizal (AM) fungi. These interactions form complex underground networks where individual plants and fungi interconnect. Despite the importance of these symbioses, research on AM fungal communities in African tropical forests remains scarce. This study investigated the AM fungi associated with ten co-occurring tree species in a Forest Reserve, Southwest Nigeria. We collected root and rhizosphere soil samples and used a metabarcoding approach to characterize fungal communities. Network analysis was then employed to examine the structure of plant-fungal associations. We identified 194 AM fungal Operational Taxonomic Units (OTUs) across six families, with the *Glomeraceae* being the dominant family. While AM fungal diversity was similar among tree species, community composition varied significantly. The network of interactions was characterized by low specialization and modularity, indicating a generalist structure. Most tree species associated with a broad range of AM fungi, and conversely, most fungi exhibited wide host breadth, linking multiple tree species. These findings suggest that AM fungi in this forest act as potential hubs in the interaction network, connecting coexisting trees and potentially contributing to ecosystem stability and function.

P.4.3. Organic amendments alter bacterial and fungal soil microbiomes over time in a subtropical banana agroecosystem. Raquel Correa Delgado (EEZ-CSIC, Spain)

Organic amendments are increasingly promoted as sustainable alternatives to intensive fertilization in banana production, yet their long-term effects on soil microbiome assembly remain poorly understood in subtropical systems. Here, we investigated how four commonly used organic amendments (chicken manure, cow manure, compost, and pelletised compost) in Canary Island influence bacterial (16S rRNA) and fungal (ITS) communities over a 20.8-month controlled banana pot experiment under subtropical conditions. Amplicon sequencing combined with multivariate analyses revealed that amendment identity, rather than organic input per se, was the primary driver of microbiome restructuring. Chicken manure induced the strongest and most persistent divergence in both bacterial and fungal communities, closely associated with increased soil conductivity and phosphorus availability. In contrast, cow manure and compost amendments promoted more gradual and progressive shifts, linked to higher organic matter and nitrogen levels. Bacterial richness increased over time under manure and compost treatments before declining towards the final sampling point, whereas fungal richness exhibited more variable temporal dynamics, particularly under compost. Redundancy analyses indicated that amendment-driven physicochemical changes acted as major ecological filters shaping community trajectories. Differential abundance analyses further highlighted amendment-specific microbial responses. Overall, our findings show that amendment quality determines the magnitude and direction of soil microbiome reassembly, with nutrient-rich inputs acting as strong ecological pulses and more balanced amendments promoting progressive community diversification in subtropical banana systems.

P.4.4. Rye diversification and organic amendments as drivers of beneficial soil microbial communities and agroecosystem resilience. Muhammad Tahir Khan (Vytautas Magnus University, Lithuania)

Soil health is fundamentally shaped by the structure and functionality of its microbial communities, which regulate nutrient cycling, plant growth, and resilience to environmental stress. Identifying agronomic strategies that enhance beneficial microorganisms is therefore central to sustainable agriculture. This study investigates how diversified rye cultivation combined with organic amendments influences soil microbial communities and ecosystem functioning under temperate agroecosystem conditions. Field experiments were established to compare monoculture rye systems with diversified rye-based cultivation under organic management. Soil physicochemical parameters, microbial activity indicators, and community composition were assessed to evaluate shifts in beneficial microbial populations. High-resolution sequencing approaches were used to characterize microbial alpha and beta diversity and to identify structural differences among management systems. Preliminary findings from this ongoing study indicate that rye diversification, particularly when combined with organic inputs, promotes greater microbial diversity and distinct community assemblages compared to monoculture systems. These changes are associated with improved nutrient dynamics and enhanced soil biological activity, suggesting strengthened ecological interactions within the rhizosphere. The results highlight the potential of integrating crop diversification and organic amendments to stimulate beneficial soil microorganisms, contributing to improved soil health and long-term agroecosystem resilience. This work aligns closely with the objectives of ROOT-BENEFIT by advancing biologically driven solutions for sustainable and climate-resilient agriculture.

P.4.5. Effects of long-term tillage and fertilisation regimes on arbuscular mycorrhizal fungi in the roots of selected crops. Irena Maček (University of Ljubljana, Slovenia)

Reduced tillage intensity is known to increase soil organic carbon in the topsoil and also benefits soil plant-beneficial arbuscular mycorrhizal (AM) fungal communities. In this study, the effects of different types of tillage (no-till and conventional mouldboard tillage) combined with three fertilisation regimes (unfertilised control, mineral NPK, and organic compost) on root colonisation by mycorrhizal fungi were examined two decades after the experiment was initiated in three crop species (*Secale cereale*, *Triticum aestivum*, and *Zea mays*). AM fungi associated with all three species responded similarly to mechanical disturbance (tillage) and fertilisation regime, with lower root fungal colonisation and arbuscule density in conventional tillage and NPK-fertilised plots. The lowest root colonisation and arbuscule density in all three crops were found in the combined tillage and NPK treatments, with the strongest response observed in *S. cereale*. Our results show a direct response of AM fungi to agricultural intensification and highlight the importance of sustainable practices with minimal soil disturbance and reduced use of mineral fertilisers.

P.4.6. Microbial inoculants in vineyards: Field impacts on grapevine performance and soil health. Sofia Pereira (Universidade Católica Portuguesa, Portugal)

Agriculture is increasingly challenged by climate change, creating an urgent need for agroecological strategies that enhance crop resilience while maintaining productivity and environmental sustainability. Among the proposed solutions, microbial inoculants have emerged as promising allies due to their potential to promote plant growth, improve nutrient availability, and strengthen plant tolerance to abiotic stress. However, field-based evidence under real vineyard conditions remains limited, particularly regarding effects on soil biological functioning and must composition. A field trial was established in the Douro wine region to evaluate the impact of selected microbial inoculants on grapevine performance, soil biodiversity and enzymatic activities, and must attributes. The study aimed to provide an integrated assessment of both agronomic and soil health responses under real vineyard conditions. Results indicated that microbial inoculation did not significantly influence grapevine productivity compared with the control. However, notable changes were observed in must composition, with inoculated treatments showing higher sugar concentrations and increased organic acid content. Soil biological activity responded positively to inoculation, as evidenced by elevated dehydrogenase and acid phosphatase activities, suggesting enhanced microbial metabolic functioning and nutrient cycling potential. In contrast, soil bacterial biodiversity did not differ between treatments, indicating that inoculation did not alter the native microbial community structure. Overall, these findings suggest that microbial inoculants can enhance soil functional activity and improve must composition without adversely affecting indigenous soil biodiversity, supporting their potential as an agroecological tool for sustainable viticulture under climate change pressures. Nevertheless, further long-term studies are required to elucidate their consistency and underlying mechanisms across diverse environmental conditions.

P.4.7. Field performance of tailored fungal–bacterial consortia against *Rhizoctonia solani* in lettuce. Giulia Capella (Research Institute of Organic Agriculture FiBL, Switzerland)

Soil-borne fungal pathogens are a major threat to high-value crops such as vegetables. Although synthetic fungicides remain the most effective control strategy, their intensive use threatens ecosystem functioning and long-term sustainability. Microbial biocontrol agents offer a promising alternative, but their effectiveness in the field is often inconsistent and requires improvement. This study aimed to enhance biocontrol efficacy by combining beneficial fungi and bacteria into tailored microbial consortia. We hypothesized that synergistic interactions, such as fungal highways, could improve establishment and pathogen suppression. Further, we are developing a novel formulation based on encapsulation of the microorganisms. Pathosystem-specific consortia were developed to support lettuce against the soil-borne pathogen Rhizoctonia solani. The fungus *Clonostachys rosea* was combined with either *Comomonas testosteroni* or *Pseudomonas entomophila*, all previously isolated from Swiss disease-suppressive soils. A second strain of *C. rosea* and *Bacillus amyloliquefaciens*, derived from the commercial products Prestop® and Amylo-X®, served as positive controls. Three field trials were conducted in autumn 2025. Two agronomic and three microbial assessments were performed to evaluate plant performance and microbial establishment. Agronomic measurements revealed neither increased biomass nor reduced disease severity in treated plots. Ongoing microbial analyses will clarify whether adjustments in inoculation method, concentration of applied microorganisms, or consortium composition are required to improve field efficacy.

P.4.8. Beyond single strains: a SynCom enhances growth, nitrogen fixation and salinity tolerance in *Medicago arborea*: Implications for sustainable forage legume production. Myrto Tsiknia (Agricultural University of Athens, Greece)

Medicago arborea is a perennial leguminous shrub native to the Mediterranean basin with significant potential for ecosystem restoration in degraded lands. Plant Growth-Promoting Bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) can enhance legume establishment through mechanisms including biological nitrogen fixation (BNF), phosphate solubilization, and the production of indole-3-acetic acid and siderophores. Insights gained from *M. arborea* inoculation studies may translate directly into improved bioinoculant strategies for sustainable *Medicago sativa* (alfalfa) and other forage legumes. Soil salinization represents a growing threat to both ecosystem function and agricultural productivity, making the development of salinity-tolerant inoculation strategies increasingly relevant. This study evaluated the effect of single and combined microbial inoculants on the growth and BNF of *M. arborea* under both control and saline conditions. Four microbial strains were tested: *Ensifer meliloti* (*rhizobium*), *Pseudomonas* sp., *Streptomyces* sp., and an AMF, along with all possible multi-strain combinations. Plant biomass, nodulation, and BNF were quantified, and a suite of biochemical markers related to plant stress responses and metabolism were assessed (pigments, sugars, free amino acids, phenolics, flavonoids, and MDA). The four-strain consortium consistently outperformed all single-strain and partial-combination treatments, being the only inoculant to simultaneously enhance all measured parameters under both saline and non-saline conditions. Biochemical analyses are currently underway and will provide mechanistic insight into the observed responses. These findings support the development of multi-species microbial consortia as effective bioinoculants, with promising applications in ecosystem restoration and potentially in sustainable forage legume crop production under stress conditions.

P.4.9. *In situ* isolation and identification of orchid mycorrhizal fungi. Kenan Turgut (Akdeniz University, Turkey)

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The natural flora of Turkey hosts 24 genera and more than 170 species with 28 endemics belong to Orchidaceae family. Almost 85% of those species bear tuber under the soil. Salep orchids are collected intensely from the wild flora of Turkey due to glucomannan contents of tubers. Every year, millions of salep orchids are destroyed and therefore their reproduction and propagation are prevented. For this reason, especially intensely harvested orchids are endangered situation. Orchid seeds do not have any endosperm and thus wild orchids germinate symbiotically with mycorrhizal fungi in nature. In terms of *in vitro* seed germination, a number of papers have been published in Turkey, but no practical application for conservation of biodiversity and cultivation has been successful. If sustainable symbiotic seed germination and cultivation of salep orchids are developed, it may relieve pressure on wild orchids. In present study, *in situ* isolation and identification of orchid mycorrhiza fungus (OMF) from various plants belonging to three orchid genera (*Anacamptis*, *Serapias* and *Himantaglossum*) have been studied. DNA analysis of fungal strains isolated from orchid roots has been completed. Thirty-two of these were identified as orchid mycorrhizal fungi. We are currently conducting culture tests to verify whether isolated OMFs promotes seed germination and seedling development in the orchid species studied.

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P.4.10. *In vivo* performance of Plant Growth-Promoting Bacteria isolated from Mediterranean soils: Pot-scale validation for biofertilizer development. Ana Sousa (Universidade Católica Portuguesa, Portugal)

Biofertilizer formulations adoption in sustainable soil management has accelerated in recent years as a strategy to reduce reliance on synthetic chemical inputs and their negative environmental impacts. Plant growth-promoting bacteria (PGPB) represent a cornerstone of these microbial inoculants, exhibiting strategies like nutrient acquisition mechanisms, phytohormone and enzymes production, biocontrol activity, that can enhance crop performance and help plants to thrive in harsh environments. Conventional biofertilizer development relies on in vitro traits screening to select candidate strains. However, laboratory performance frequently fails to predict field efficacy. This lab-to-field gap stems primarily from the absence of in vivo validation under realistic soil and environmental conditions. Addressing this bottleneck requires, as a first step, controlled pot-scale experiments that establish the bridge between in vitro promise and field deployment. An in vivo performance validation of, approximately, twenty PGPBs isolated from soil samples collected in Mértola (Alentejo, Portugal) was performed. A pot experiment (n = 4), using non-sterile soil and a *Triticum aestivum* L. target crop variety was conducted to assess plant response to bacterial inoculation, under three irrigation regimes (80%, 40%, and 20% of soil water holding capacity). Shoot and root dry biomass, shoot elongation, photosynthetic capacity (SPAD), and proline content analysis are ongoing. Soil samples were collected at sowing, post-inoculation, and harvest and will undergo microbial community profiling, aggregates stability, and macro and micronutrient bioavailability. The expected outcomes will help elucidate critical determinants of biofertilizer efficacy, including the influence of native soil microbiota, physicochemical and abiotic conditions context.

P.4.11. Ectomycorrhizal inoculation for improved tree seedling performance in degraded soils. Jelena Lazarević (University of Montenegro, Montenegro)

Mycorrhizal colonization of seedling roots is a key determinant of successful forest restoration, particularly in degraded, nutrient-poor soils and under harsh environmental conditions. Our recent research on the effects of forest fires on soil fungal communities at the upper tree line demonstrated long-term alterations in fungal community structure, accompanied by significant changes in soil nutrient dynamics. Beyond fire disturbance, many forest ecosystems are similarly affected by other anthropogenic pressures, which can disrupt soil microbial communities and impair natural regeneration processes. These findings show the renewed importance of targeted mycorrhization of forest seedlings as a strategy for the restoration of degraded and marginal habitats. We developed and evaluated efficient methods for ectomycorrhizal (ECM) inoculation of coniferous seedlings commonly used in reforestation programs in Western Balkan countries. The inocula, both vegetative (mycelial) and spore-based, were derived from fungal species collected in native forests exposed to harsh environmental conditions. Fungal species selection was guided by ecological suitability and the practical feasibility of ECM inoculum production. Inoculum production and performance were assessed under controlled laboratory conditions and subsequently validated in forest nurseries. Laboratory optimization focused on improving ECM culture establishment and growth efficiency. Due to its simplicity of application and higher colonization efficiency, spore inoculum proved to be the most effective approach. More recently, we extended this methodology to deciduous tree species, including oaks. In addition to assessing initial colonization rates, we evaluated the persistence of established mycorrhizae following outplanting in brown acidic soils with contrasting land-use histories. Furthermore, we examined how pre-inoculation influenced the subsequent development and succession of root-associated fungal communities after field establishment

P.4.12. Biocontrol metabolites of *Aspergillus piperis* A/5 – Possibilities and challenges for application. Jelena Jovičić-Petrović (University of Belgrade, Serbia)

A previous study on the biocontrol mechanisms of *Aspergillus piperis* A/5 against *Pythium aphanidermatum* revealed an unusual outcome of organic solvent extraction of the liquid culture filtrate. The antifungal activity of the liquid culture filtrate remained in the aqueous solution, and was attributed to proteins resistant to heat, organic solvents, and proteinase K. A significant amount of protein was detected by SDS-PAGE in the culture filtrate. In the current study, amino-acid analysis was performed using ion-exchange chromatography with an automatic AA analyser Biochrom 30+. The results showed that glutamic and aspartic acids dominate in the mixture, followed by glycine, proline, lysine, and alanine. The amino-acid content indicates the possibility of using *A. piperis* A/5 both as biopesticide and biostimulant product, since the beneficial effects of amino-acid application in agriculture have been proven, particularly for the amino acids that were more abundant in the culture filtrate. Besides biocontrol proteins, a complex mixture of *A. piperis* A/5 metabolites is rich in organic acids, which may represent one of the main challenges for application. The culture filtrate after seven days of incubation is characterized by a low pH value, between 1.8 and 2.3. The possibility of raising the pH before application is among options, which required research to optimize the dilution, pH value, and manage the excess of sodium caused by neutralization, aimed to avoid harmful effects on plants. The preliminary results obtained through germination tests indicate that appropriate optimization of bioproduct is crucial to maximize its beneficial effects for application in agriculture.