Cádiz

XX SEFIN Congress

III BeMiPlant

Beneficial Plant-Microbe Interactions

26-27-28/MAYO/2026

XX Congreso de la Sociedad Española de Fijación de Nitrógeno

III Congreso Beneficial Plant-Microbe Interactions

Cádiz

SEFIN Congress

BeMiPlant

Beneficial Plant-Microbe Interactions

26-27-28/MAYO/2026

ABSTRACT BOOK

Dear colleagues,

We are pleased to invite you to the 20th Congress of the Spanish Society for Nitrogen Fixation (SEFIN), which will be held in Cádiz from May 26 to 28, 2026.

This ancient city, with more than three thousand years of history, has always been a meeting point for cultures and ideas - a true melting pot that perfectly reflects the spirit of our congress: a space for the exchange of ideas, dialogue among colleagues, and the strengthening of collaborations. We believe that Cádiz offers an outstanding setting to host this new edition of the SEFIN congress.

As you know, in recent years the focus of the congress has evolved from the study of symbiotic nitrogen fixation toward a broader scope encompassing all beneficial interactions between plants and microorganisms, greatly expanding opportunities for collaboration and scientific innovation. In this context, the congress will also be the third edition of BeMiPlant (Beneficial Plant-Microbe Interactions), thereby consolidating this dual dimension of the meeting.

The organizing team sincerely thanks SEFIN for the trust placed in us to carry out such a special event.

It is therefore both an honor and a great pleasure to invite you to participate in the 20th SEFIN Congress / 3rd BeMiPlant. We are confident that, as in previous editions, it will be an enriching experience both scientifically and personally.

The Organizing Committee

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Sede para Secretaría del Congreso, las pausas para café, los almuerzos y la sesión de pósteres:
Edificio Constitución de 1812 (Antiguo Cuartel La Bomba)
Paseo Carlos III. nº 3, 11003 Cádiz

Sede para actos de presentación y de clausura, conferencias, sesiones orales y asamblea SEFIN:
Salón de Grados, Facultad de Medicina, UCA
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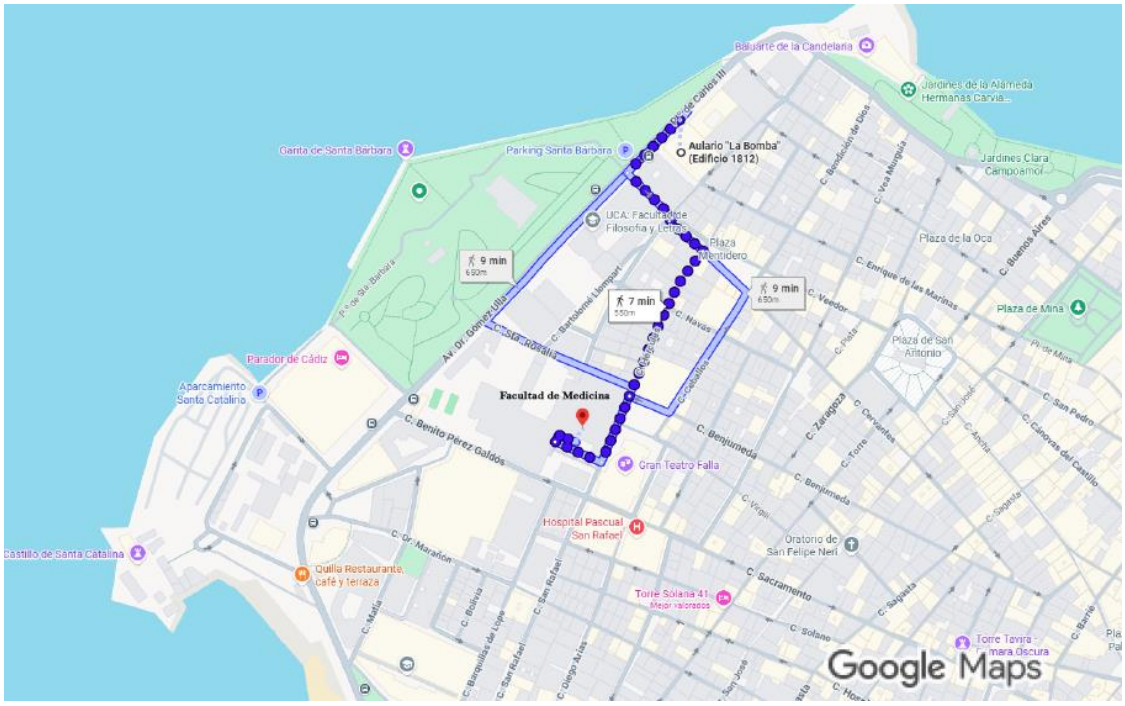

Edificio Constitución de 1812

Facultad de Medicina

Welcome cocktail
(Parador de Cádiz)

Gala dinner
(Baluarte de los Mártires)

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SCIENTIFIC PROGRAM

Congress Secretariat, coffee breaks, lunches, and the poster session will be held in the **Edificio Constitución de 1812**. SEFIN Assembly, Welcome and Closing Ceremonies, Opening, Closing and Palomares conferences, Oral and Flash talks will be held in the **Salón de Grados (Facultad de Medicina)**.

Tuesday, 26th May 2026

15:00–17:00	Registration *(Edificio Constitución de 1812)*
17:00–18:00	SEFIN Assembly *(Facultad de Medicina)*
18:15–18:45	Welcome Ceremony *(Facultad de Medicina)*
18:45–19:45	Opening Conference *(Facultad de Medicina)*

Francisco Miguel Cornejo Castillo (Institut de Ciències del Mar, CSIC)

"The nitroplast: the first known nitrogen-fixing organelle in eukaryotes"

20:00	Welcome Cocktail *(Parador de Cádiz)*

Wenesdey, 27th May 2026

9:30–11:10 Session 1: Biological Nitrogen Fixation. Diversity of beneficial microorganisms for plants. *Facultad de Medicina.*

Chairs: **Juan Ignacio Vílchez (GREEN-IT, Univ. Nova de Lisboa)**

Esther Menéndez (Univ. Salamanca)

Oral presentations

9:30-9:50 **O1.1. Flores-Félix JD**, Igual M, García Fraile P.
"Differential modulation of rhizosphere microbial communities by *Mesorhizobium* and *Bradyrhizobium* symbionts of *Lotus corniculatus*"

9:50-10:10 **O1.2. Jacott CN**, Lopez-Beltran M, Thuss R, Cutiño-Gobea A, Manfrin TP, Requena P, de la Peña M, Fuentes-Romero F, Acosta-Jurado S, Espuny MJ, Ollero FJ, Vinardell JM, López-Baena FJ, del Cerro P.
"Natural genetic variation in soybean identifies a novel locus restricting symbiosis with rhizobia"

10:10-10:30 **O1.3. Mariscal V**, Paganelli A, Sánchez del Solar C, Álvarez C.
"Dependence of the CSSP signalling pathway for endophytic colonization of rice by *Nostoc punctiforme*"

Flash Talks

10:30-10:35 **F1.1. Collantes-García JA,** Rosa-Núñez E, Armas AM, Raimunda C, Pérez-González A, Guo Y, Echávarri-Erasun C, Rubio LM, González-Guerrero M.
"*Azotobacter vinelandii* glutaredoxin D delivers the core [Fe_2S_2] cluster to nitrogenase cofactor scaffold protein NifU"

10:35-10:40 **F1.2.** Domingo-Serrano L, De Sousa BFS, Romero-Ulloa A, Sánchez-Crespo M, Cuesta-Morrondo S, Cubero J, Alvarez-Martinez C, Palacios JM, **Rey L.**
"Analysis of the role of rhizobial type VI secretion systems in microbial competition"

10:40-10:45 **F1.3. Pérez-González A,** Martin Del Campo JS, Dean DR.
"*In vivo* production of hybrid nitrogenases in *Azotobacter vinelandii*"

10:45-10:50 **F1.4. Pérez-González S,** Montañez M, De Ron AM, Rodiño AP, Vílchez JI, Delgado MJ, Tortosa G.
"The endophytic role and growth-promoting ability of *Burkholderia alba* in *Rhizobium leucaenae*-common bean symbiosis"

10:50-10:55 **F1.5. Santiesteban-Serrano A,** Karst J, Rincón A, Aponte C.
"A global meta-analysis of soil fungal responses to changing fire regimes in ectomycorrhizal-dominated forests"

10:55-11:00 **F1.6. Thuss R**, Lopez-Beltran M, Cutiño-Gobea A, Manfrin TP, Requena P, de la Peña M, Fuentes-Romero F, Acosta-Jurado S, Espuny MJ, Ollero FJ, Vinardell JM, López-Baena F, del Cerro P, Jacott CN.
"Unlocking the genetic basis of soybean-rhizobia compatibility through GWAS in wild populations"

11:20–12:00 **Coffee break.** *Edificio Constitución de 1812.*

12:10–13:50 Session 2: Molecular Biology and Physiology of Plant–Microorganism Interactions (I). *Facultad de Medicina.*

Chairs: **Catherine N. Jacott (Univ. Sevilla)**

Marta Albareda (CBGP-UPM-INIA)

Oral presentations

12:10-12:30 **O2.1. Arbelo-Brito G,** Gonzáles-Dominici LI, Ayala-García P, Borrero-de-Acuña JM, Pérez-Montaño F, García Fraile P, Saati-Santamaría Z.
"Peptidoglycan remodelling in *Pseudomonas* modulate host perception, defense activation and root hair development"

12:30-12:50 **O2.2.** Coca V, Arribas-Hernandez L, Pozueta-Romero J, **Morcillo, RJL.**
"m^{6}A RNA methylation enables plant growth responses to microbial volatiles by regulating growth-defense balance"

Flash Talks

12:50-12:55 **F2.1. Gómez-Fernández GO**, Huisman R, Frances L, Zamarreño ÁM, García-Mina JM, Arrese-Igor C, de Carvalho-Niebel F, Kohlen W, Larrainzar E.
"NIN connects Nod factor signalling and ethylene biosynthesis in *Medicago truncatula*"

12:55-13:00 **F2.2. Carvia-Hermoso C**, Cuéllar V, del Cerro P, Vinardell JM, van Dillewijn P, Soto MJ.
"Functional characterization of a RelA-associated toxin-antitoxin system in *Sinorhizobium meliloti*"

13:00-13:05 **F2.3. Cutugno L,** Gigli A, Aguilar De Prada SS, Rubio LM.
"Engineering nitrogenase electron-transport component in *Saccharomyces cerevisiae*"

13:05-13:10 **F2.4.** Marqués-Gálvez JE, Navarro-Ródenas A, Arenas F, Guarnizo-Serrudo A, Andreu-Ardil L, Fernández-Cortés A, Gazquez F, Andrino A, Querejeta JI, **Morte A.**
"Elemental and isotopic characterization of *Terfezia claveryi* Chatin reveals key nutritional traits, trophic lifestyle and water use of desert truffles"

13:10-13:15 **F2.5. García-Díaz, I**, García-Calderón M, Betti M, Márquez AJ.
"Studies of differentially expressed genes in the *Lotus japonicus – Mesorhizobium loti* symbiosis"

13:15-13:20 **F2.6. García-Rodríguez D,** Ayala-García P, Ollero FJ, Borrero de Acuña JM, Vinardell JM, López-Baena FJ, Jiménez-Guerrero I.
"*EveD:* an extracellular vesicle-secreted effector in rhizobia"

13:30-13:50 **Conference by "Biomol" and "Fundación Instituto Biomol"**

14:00–15:50 **Lunch Break.** *Edificio Constitución de 1812.*

16:00–17:40 Session 3: Molecular Biology and Physiology of Plant-Microorganism Interactions (II). *Facultad de Medicina.*

Chairs: **Estíbaliz Larrainzar (Univ. Pública de Navarra)**

María José Soto (EEZ-CSIC)

Oral presentations

16:00-16:20 **O3.1.** Navarro-Gómez C, Collantes-García JM, Rodríguez-Simón M, Imperial J, Escudero V, **González-Guerrero M.**
"Unveiling new metalloproteins in *Medicago truncatula* nodules"

16:20-16:40 **O3.2. Pérez-Mendoza D**, Ruiz-Sáez L, Marchante JA, Muñoz S, Pacheco PJ, Peinado J, Lloret J, Rodríguez-Carvajal MA, Sanjuán J.
"Bioprospecting c-di-GMP activated exopolysaccharides in beneficial plant-interacting bacteria"

16:40-17:00 **O3.3. Jiménez-Zurdo JI,** García-Tomsig N, Guedes-García SK, Robledo M.
"Multifaceted regulation of legume nodulation by the rhizobial small RNA NfeR1"

Flash Talks

17:00-17:05 **F3.1. Aranda-Pérez J,** Sánchez-Aguilar MC, Cutiño-Gobea AM, Pérez-Montaño F, Medina C.
"Cyclic di-GMP Modulation of Quorum Sensing and Its Impact on Type VI Secretion System Function in *Sinorhizobium fredii*"

17:05-17:10 **F3.2. González-Dominici LI**, Yeshvekar R, Benitez-Alfonso Y, Saati-Santamaría Z, García-Fraile P.
"Microbial and light signalling converge to shape root system architecture"

17:10-17:15 **F3.3. Herrero-Gómez I,** Krenz B, Ayala-García P, Jiménez-Guerrero I, Pérez-Montaño F, Borrero-de Acuña JM.
"Engineering bacterial extracellular vesicles as tools for intracellular effector delivery and activation of the plant immune system."

17:15-17:20 **F3.4. Jiménez-Leiva A,** Cabrera JJ, García-Pedrosa MA, Mesa S, Delgado MJ.
"The *Bradyrhizobium diazoefficiens* transcriptome in response to copper starvation under denitrifying conditions: Insights into the FixLJ-FixK2 and RegSR-NifA regulatory cross-talk"

17:20-17:25 **F3.5. Kozikova D,** Martínez-Lüscher J, Torrens J, Antolín MC, Gogorcena Y, Goicoechea N, Pascual I.
"Cultivar-specific flavonoid responses to arbuscular mycorrhizal inoculation under projected climate change scenarios"

17:25-17:30 **F3.6. Payá-Tormo L1,** Echavarri-Erasun C, Makarovsky-Saavedra N, Pérez-González A, Yang ZY, Guo Y, Seefeldt LC, Rubio LM.
"FeMo-cofactor synthesis by a thermophilic nitrogenase lacking the NifEN scaffold"

17:50–19:20 **Posters session with "piscolabis".** *Edificio Constitución de 1812.*

19:30–21:00 **Guided visit.** *Edificio Constitución de 1812.*

— Free time for dinner

Thursday, 28th May 2026

9:30–11:10 Session 4: Agronomic and Ecological Uses of Plant–Microorganism Interactions. *Facultad de Medicina.*

Chairs: **Carmen Sánchez Cañizares (IRNASA-CSIC)**

Rafael Rivilla (Univ. Autónoma de Madrid)

Oral presentations

9:30-9:50 **O4.1. García-Toledo M**, Amador-Laguna FJ, Rodelas B, Pozo C, Purswani J.
"Evaluation of Social PGPR SynComs on Common Greenhouse Crops under Climatic Stress"

9:50-10:10 **O4.2. Lidoy J,** Minchev Z, España-Luque L, Benítez-González AM, Ramos A, García J, Berrio E, Nesterenko O, Díaz-Ortiz P, Meléndez-Martínez AJ, Pozo MJ, López-Ráez JA.
"Use of arbuscular mycorrhizal fungi as biostimulants for carotenoid biofortification in tomato fruits under field conditions"

10:10-10:30 **O4.3. Carola JM**, Vílchez JI.
"Synthetic Assemblies for Microbiome-Based Agriculture (SAMBA): a trait-based framework for predicting and designing microbial consortia"

Flash Talks

10:30-10:35 **F4.1. Escudero-Martinez C**, Browne E Y, Schwalm H, Brown M, Brown L, Roberts D, Morris J, Hedley P, Thorpe P, Abbott J, Brennan FP, Bulgarelli D, George TS, Oburger E.
"Evaluation of combined root exudates and rhizosphere microbiome sampling to elucidate rhizosphere traits"

10:35-10:40 **F4.2. Shahid I**, Arrese-Igor C, Mehnaz, Larrainzar E.
"Metabolomic and genomic characterization of *Pseudomonas chlororaphis* strains for biofungicide applications"

10:40-10:45 **F4.3. Majo-Cuervo M**, Saati-Santamaría Z, García-Fraile P, diCenzo G, Mateos PF, Menéndez E.
"Different strategies for synthetic community design drive distinct rhizoplane colonization and plant growth outcomes in the wheat rhizoplane"

10:45-10:50 **F4.4. McQuade MR**, Silva D, Vílchez J.I
"Algal extracts stimulate plant growth-promoting bacteria and enhance tomato growth through microbial biostimulation"

10:50-10:55 **F4.5. Mazuecos-Aguilera I**, Anta-Fernández F, March-Ballester V, López-Bornay P, González-Andrés F.
"Impact of PGPR addition to compost on nutrient use efficiency by crops and soil microbiome composition"

10:55-11:00 **F4.6. Pajuelo E,** Domínguez-Pérez GM, Mateos-Naranjo E, Redondo-Gómez S, Rodríguez-Llorente ID.
"Inter-domain plant-*Archaea* interactions: halophilic archaea as biofertilizers in saline soils?"

11:20–12:00 **Coffee break.** *Edificio Constitución de 1812.*

12:10–13:50 Session 5: Effects of Biotic and Abiotic Stresses on Beneficial Plant–Microorganism Interactions. *Facultad de Medicina.*

Chairs: **Isabel Videira (INIA)**

María Camacho (IFAPA)

Oral presentations

12:10-12:30 **O5.1. Gómez-Morte A**, Albó D, Oliach D, Bonet JJ, Ferrio JP, Peguero-Pina JJ, Rincón A.
"Black truffle mycelium growth and host specific antioxidant response under combined atmospheric and soil drought stress"

12:30-12:50 **O5.2. Niraula K**, Vílchez JI.
"Stress-induced root exudates recruit beneficial microbes and enhance stress tolerance in tomato"

12:50-13:10 **O5.3. Vergara A,** Martín L, López-García A, Ochoa-Hueso R, Carrascosa A, Alarcón MV, Alguacil MM.
"Arbuscular mycorrhizal fungal communities show greater resilience to climate change under organic management, enhancing soil multifunctionality in Mediterranean vineyards"

Flash Talks

13:10-13:15 **F5.1. García-Chávez, L**, Igual JM, Amin H, Niño-Sánchez J, Díez-Casero JJ, Valverde A.
"Responses of non-target soil microbial communities to double-stranded RNA application"

13:15-13:20 **F5.2. García-Gómez M**, Saati-Santamaría Z, Castro JF, Gasmi M, Menéndez E, Carro L.
"Environmental drivers of fungal community diversity: a comparative ITS-based study across desert and temperate habitats"

13:20-13:25 **F5.3. Ortega-Chávez J,** Majo-Cuervo M, Menéndez E, Olmo R.
"Plant-associated bacteria with nematicidal potential: a sustainable biocontrol strategy"

13:25-13:30 **F5.4. Piroli C**, Lendínez S, Cáceres N, Garrido JL, Azcón-Aguilar C, Ferrol N, López-García A.
"Drivers of arbuscular mycorrhizal fungal communities in Mediterranean agroecosystems under global change"

13:30-13:35 **F5.5. Poveda J**, Velasco P.
"A new way to help their colleagues against the pathogen: *Trichoderma hamatum* induces plant systemic defenses by releasing elicitors from the *Sclerotinia sclerotiorum* cell wall"

13:35-13:40 **F5.6. Sousa A**, Vílchez JI.
"Halophilic bacteria from Iberian salt flats enhance crop tolerance to salinity stress"

14:00–15:50 **Lunch break.** *Edificio Constitución de 1812.*

16:00–17:00 Palomares Award Conference *(Facultad de Medicina)*

Pablo del Cerro (Univ. Sevilla)
"The molecular dialogue in rhizobia-legume symbiosis"

17:00–18:00 Closing Conference *(Facultad de Medicina)*

Sofie Goormachtig (Univ. Ghent)
"Rooted Partnerships: From Microbial Symbiosis to Climate-Ready Legumes, a High-Resolution Headshot."

18:00–18:30	Closing Ceremony *(Facultad de Medicina)*
21:00	Gala Dinner *(Restaurante Baluarte de los Mártires)*

OPENING CONFERENCE

The nitroplast: the first known nitrogen-fixing organelle in eukaryotes

Cornejo-Castillo, F.M.[1*]

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Abstract

Nitrogen (N) is essential for life, and although N_2 is the main component of the atmosphere, it is inaccessible to most living organisms. An exception to this rule is represented by certain nitrogen-fixing bacteria and archaea (diazotrophs), which have the ability to transform N_2 into bioavailable forms. Historically, eukaryotic organisms were thought to be unable to fix N_2, although they could establish symbiotic relationships with diazotrophs, thereby gaining access to this essential resource. Among the diversity of N_2-fixing organisms in marine environments, the diazotrophic cyanobacterium *Candidatus* Atelocyanobacterium thalassa (also known as UCYN-A) lives in symbiosis with certain haptophyte microalgae closely related to the species *Braarudosphaera bigelowii*. Recently, UCYN-A has been shown to have evolved from an endosymbiont into an organelle, making *B. bigelowii* the first known N_2-fixing eukaryotic species to date. In this presentation, I will describe the key lines of evidence that have led to the discovery of this new eukaryotic organelle: the nitroplast.

References

[1] Cornejo-Castillo, F.M. *et al*. Cell, 2024, 187, 1762-1768.
[2] Coale, T.H. *et al*. Science, 2024, 384, 217-222.
[3] Massana, R. Science, 384, 160-161.

The molecular dialogue in rhizobia-legume symbiosis

del Cerro, P.[1*]

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Abstract

Symbiotic nitrogen fixation between rhizobia and legumes depends on a precise molecular dialogue. Rhizobia sense flavonoids released by plant roots, which activate the transcriptional regulator NodD1 and trigger the synthesis of Nod factors, bacterial signaling molecules that the plant recognizes as a symbiotic partner.

My work demonstrated that Nod factor synthesis is induced in the presence of flavonoids, as well as osmotic-stresses, and upon specific regulation of NodD1 and NodD2, respectively [1]. NodD2 is constitutively active, and its expression alone is sufficient to drive Nod factor synthesis [2]. Our preliminary data suggest that this activation could be important during rhizobial root colonization.

At the plant epidermis level, the recognition of Nod factors activates the Common Symbiotic Signaling Pathway, characterized by a calcium oscillation event in the nucleus. These calcium oscillations are regulated by the action of the ion channel CNGC15 [3]. My work has demonstrated that Calmodulin2 regulates CNGC15 activity [4]. Additionally, a gain-of-function mutation in CNGC15 makes the channel constitutively active, generating calcium oscillations and nodule organogenesis even without rhizobia, and this mutation enhance rhizobia symbiosis [5].

However, triggering the symbiotic program is not sufficient for successful colonization. Like pathogens, rhizobia use Type III Secretion Systems to inject effectors into plant cells and suppress defence responses. We have identified novel effector targets in soybean using Yeast Two-Hybrid screening, which are currently being validated in planta. In parallel, our genome-wide association study across 300 soybean genotypes has revealed a novel resistance gene that controls compatibility with rhizobia.

References

[1] del Cerro et al. ***Scientific Reports***. 2017. 7 (1), 46712.
[2] Ayala-García et al. ***Journal of Experimental Botany***. 2022. 73 (19), 6931-6941.
[3] Jacott & del Cerro. ***Journal of Experimental Botany***. 2024. 75 (22), 6998-7005.
[4] del Cerro P et al. ***PNAS***. 2022. 119(13), e2200099119.
[5] Cook et al. ***Nature***. 2025. 638 (8051), 752-759.

Funding

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CLOSING CONFERENCE

Rooted Partnerships: From Microbial Symbiosis to Climate-Ready Legumes

Goormachtig, S. [1*]

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Abstract (300 words maximum)

Our research focuses on beneficial plant–microbe interactions to develop biotechnological solutions for sustainable agriculture. We investigate legume–rhizobia symbiosis, a process in which root nodules host nitrogen-fixing bacteria that naturally reduce the need for synthetic fertilizers. My group currently leads several initiatives aimed at establishing soybean as a climate-resilient crop in North-Western Europe. A key part of this work is identifying native nitrogen-fixing rhizobial strains adapted to colder soils. This effort includes the citizen-science initiative “Soy in 1000 Gardens,” which connects our research with public engagement and real-world field observations. We combine these community-generated data with advanced plant omics technologies to bridge fundamental biological insights with practical, sustainable agricultural applications, ultimately contributing to more resilient cropping systems.

SESSION 1

Biological Nitrogen Fixation. Diversity of beneficial microorganisms for plants.

ORAL PRESENTATIONS

O1.1

Differential modulation of rhizosphere microbial communities by *Mesorhizobium* and *Bradyrhizobium* symbionts of *Lotus corniculatus*

Flores-Félix, J. D.[1,2*], Igual, M.[3,4], García Fraile, P.[1,4]

[1]Departamento de Microbiología y Genética, Universidad de Salamanca, Spain, [2]Institute for Agribiotechnology Research (CIALE), Universidad de Salamanca, Villamayor, Salamanca, Spain [3]Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC), [4] Unidad Asociada Grupo de Interacción Planta-Microorganismo, Universidad de Salamanca - IRNASA-CSIC, Salamanca, Spain.
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Abstract

Traditionally, *Lotus corniculatus* has been regarded as a specialist legume whose endosymbionts were strictly identified within the genus *Mesorhizobium*. However, research over the past decade has challenged this paradigm [1], revealing that this species can interact with different endosymbionts across multiple genera. In this study, we conducted a comparative analysis of *L. corniculatus* symbionts isolated from two distinct forest ecosystems in the province of Salamanca: a holm oak forest (*Quercus rotundifolia*) and a Pyrenean oak forest (*Q. pyrenaica*) evaluating its ability to recover fire-affected soils. Phylogenomic characterization revealed that isolates from the holm oak forest belong to the genus *Mesorhizobium* but do not align with any currently described species, whereas those from the Pyrenean oak forest were identified as *Bradyrhizobium rifense*. Further analysis of the *nodC* symbiotic gene demonstrated that the *Mesorhizobium* strains belong to symbiovar loti, while the *Bradyrhizobium* strains correspond to symbiovar genistearum. Inoculation assays indicated distinct histological patterns in nodule development and symbiosome occupancy, with symbiovar loti exhibiting significantly higher occupancy levels. Although under axenic conditions no statistically significant differences were observed in vegetative parameters such as shoot length or biomass. However, microcosm experiments conducted employing a forest fire-affected soil from Salamanca (Spain) revealed higher efficiency in the *Mesorhizobium* genus and significant shifts in rhizospheric microbial populations. Specifically, *Mesorhizobium* inoculation led to a marked reduction in fungal (p=0.0017) and archaeal (p<0.001) diversity, while bacterial diversity remained stable (p=0.34). Taxa such as *Ktedonobacteraceae*, *Mortierellaceae*, and *Nitrosotaleaceae* showed strong correlations with *Mesorhizobium* inoculation, whereas *Aspergillaceae*, *Acetobacteraceae*, and *Burkholderiaceae* were associated with the *Bradyrhizobium* inoculum, although it was associated with a reduction in soil microbial biomass (p<0,001). Furthermore, UHPLC-Q-TOF/MS analysis of root exudates revealed significant differences in compound composition and abundance depending on the endosymbiont inoculation, suggesting that the rhizobia-legume interaction extends beyond plant fitness to profoundly influence soil microbial ecology.

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O1.2

Natural genetic variation in soybean identifies a novel locus restricting symbiosis with rhizobia

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Abstract

Symbiosis between soybean (*Glycine max*) and rhizobia is essential for **biological itrogen fixation** and offers a sustainable alternative to synthetic fertilizers. This interaction relies on mutual recognition between plant and bacteria. Rhizobia effectors delivered via the type III secretion system play a key role in this process by either promoting or restricting symbiosis depending on the plant genotype [1,2]. Despite this, the host plant genetic factors underlying these responses remain largely unknown.

To identify these determinants, we performed high-throughput phenotyping, whole-genome sequencing, and a **genome-wide association study (GWAS) of a globally diverse soybean panel** (286 accessions) inoculated with rhizobia *Sinorhizobium fredii* HH103. We observed strong genotype-dependent variation in nodulation (**Fig. 1A**) and identified a locus on chromosome 8 associated with restriction of nodulation (**Fig. 1B**). This locus contains a **candidate NLR gene** (nucleotide-binding leucine-rich repeat receptor), a class of intracellular immune receptors [3]. Two major haplotypes at this NLR were associated with nodulation, with haplotype 1 showing ~40% lower nodulation than haplotype 2 (**Fig. 1C**), implicating this locus in symbiotic restriction.

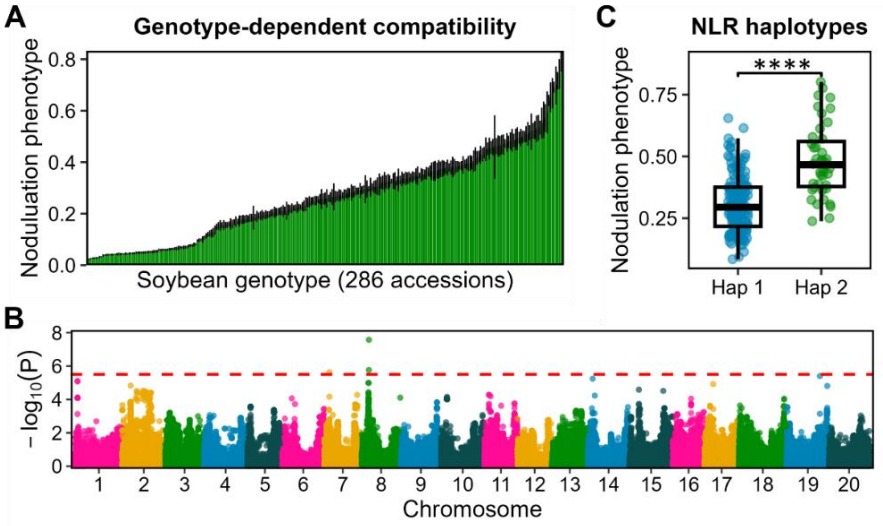

Figure 1 (**A**) Nodulation (nodule weight per plant) across 286 soybean accessions, shows strong genotype-dependent variation. (**B**) GWAS identifies a significant locus on chromosome 8. (**C**) Haplotype analysis of a candidate NLR gene shows allelic variation associated with nodulation phenotype.

Given that NLRs are common targets of microbial effectors, **we hypothesize that this NLR mediates the recognition of a rhizobia effector** [4]. Ongoing interactomics approaches (Y2H and IP-MS) aim to identify the specific effector(s) involved and define the role of this NLR in host signaling pathways.

Overall, our results identify a previously unknown genetic component controlling symbiotic compatibility, provide **new insights into plant–rhizobia coevolution**, and highlight this locus as a potential target for crop breeding to enhance symbiosis.

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O1.3

Dependence of the CSSP signalling Pathway for endophytic colonization of rice by *Nostoc punctiforme*

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Abstract

Nitrogen-fixing cyanobacteria of the order Nostocales establish symbiotic associations with a wide range of plant species, exhibiting remarkable symbiotic competence. A single cyanobacterial strain can form biological nitrogen-fixing (BNF) relationships with multiple hosts, highlighting the versatility of these interactions. Our research explores the diversity and molecular basis of cyanobacteria–plant symbioses using *Oryza sativa* and *Nostoc punctiforme* as model organisms, with particular emphasis on early colonization events and the signaling pathways required for symbiotic establishment [1].

We developed a laboratory protocol to assess the symbiotic competence of *N. punctiforme*. Three-dimensional confocal microscopy of *O. sativa* roots revealed intracellular colonization of the epidermis and exodermis. Upon entering plant tissues, *N. punctiforme* differentiates into heterocysts, the specialized cells responsible for nitrogen fixation [2]. Multi-omic analyses during the pre-symbiotic stage uncovered extensive molecular reprogramming in both partners, involving chemical signaling, surface remodeling, and adhesion processes, and provide strong evidence for the participation of the Common Symbiosis Signaling Pathway (CSSP) [3].

To further dissect this communication, we generated *N. punctiforme* mutants impaired in Nod-factor biosynthesis. These mutants display reduced colonization of *O. sativa* roots, reinforcing the involvement of CSSP-mediated signaling. Complementarily, *O. sativa* mutants defective in core CSSP genes—CYCLOPS, CAMK, and POLLUX—exhibit impaired symbiotic responses and diminished cyanobacterial colonization, confirming that canonical CSSP components are required for the establishment of these cyanobacteria–plant interaction.

Together, these findings advance our understanding of the molecular dialogue underlying nitrogen-fixing cyanobacterial symbioses and offer promising avenues for the development of BNF-based biotechnological applications.

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SESSION 1

Biological Nitrogen Fixation. Diversity of beneficial microorganisms for plants.

FLASH PRESENTATIONS

F1.1

Azotobacter vinelandii glutaredoxin D delivers the core [Fe_2S_2] cluster to nitrogenase cofactor scaffold protein NifU

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Abstract

The scaffold protein NifU plays a central role in assembling the precursor [Fe_4S_4] clusters required for nitrogenase to function [1]. The synthesis of these precursors depends on a catalytic [Fe_2S_2] group within NifU core ferredoxin domain. Here, we show that the monothiol glutaredoxin GrxD delivers this cluster to the NifU scaffold protein. Consistently, *grxD* mutants have reduced nitrogenase activity, the result of altered iron allocation to this enzyme. Biochemical assays show that GrxD unidirectionally transfers [Fe_2S_2] to NifU through protein-protein interaction. This allows GrxD to restore apo-NifU functionality, enabling proper [Fe_4S_4] synthesis, and NifH activation. These findings are crucial to understand how iron is allocated to nitrogenase for biological nitrogen fixation.

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F1.2

Analysis of the role of rhizobial type VI secretion systems in microbial competition

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Abstract

The type VI secretion system (T6SS) has been described primarily in the phylum *Pseudomonadota* and can inject proteins called effectors into bacteria and eukaryotic cells. *Rhizobium* T6SS has been reported to influence symbiosis with legumes in positive, neutral or negative ways [1]. In this communication we evaluate the role of rhizobial T6SSs in microbial competition. We analyze two bean nodulating strains, *Rhizobium etli* (*Re*) Mim1 encoding a T6SS (T6SS+) and *Re* CFN42 (T6SS-) in free-living and nodule competitiveness. It was observed that these bacteria do not compete with each other; however, in the competition assay for nodule colonization, CFN42 occupied 70% of the nodules at a 1:1 ratio and 55% at a 1:10 ratio compared to Mim1. Interbacterial competitiveness for growth of Mim1 and T6SS mutants against *E. coli*, was also tested and no competition was detected at different ratios. Additionally, competition assays using Mim1 and T6SS mutant and *Xanthomonas* strains were performed. Assays of coincubation of *R. etli* and *X. citri* 306 did not show competence or relevance of the T6SS in their contact-dependent coexistence. Other trials with *Xanthomonas* strains that infect bean are currently underway. We are also conducting experiments involving three pea nodulating strains: *R. leguminosarum* UPM791 (T6SS-), *R. ruizarguesonis* UPM1134 and *R. leguminosarum* FRP3G5 with T6SSs which are markedly different, as they have an amino acid conservation of about 30% and very different gene organization. Finally the effect of the T6SS on interaction with amoebae was assessed with *Dictyostelium discoideum* and rhizobial strains as Mim1, Mim1 T6SS mutants, etc. *D. discoideum* was spotted onto pre-established bacterial lawns and examined at 24 h and 72 h post-inoculation. Preliminary results suggested that Re Mim1 T6SS activity facilitates predation by *D. discoideum,* perhaps linked to the lower level of exopolysaccharide production.

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F1.3

In vivo production of hybrid nitrogenases in *Azotobacter vinelandii*.

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Abstract

Azotobacter vinelandii is an aerobic free-living diazotroph that produces the three nitrogenase isoforms, which are genetically distinct but functionally and mechanistically similar. These isoforms are designated Mo-, V-, and Fe-only nitrogenases according to the metal composition of their associated active site cofactors[1-3]. Formation of FeMo-co and FeV-co, the active site cofactors of the Mo- and V- nitrogenases, occur on the NifEN and VnfEN scaffold proteins, respectively, upon which an apical Fe atom within NifB-co is replaced by Mo or V and the organic ligand homocitrate is attached[4]. In contrast, formation of FeFe-co does not require a molecular scaffold[4]. Differential expression of the nitrogenase isoforms in response to a physiological demand for fixed nitrogen and to the availability of Mo or V is subjected exquisite regulation. However, physiological transitions between conditions favoring the accumulation of the different isoforms could result in the incorporation of an incorrect cofactor, rendering inactive hybrid species. In a previous work, we reported the *in vivo* production of hybrid Fe-only nitrogenases containing either FeMo-co and FeV-co at scale[5]. In this communication, we report the *in vivo* production of Mo-nitrogenase containing FeV-co and V-nitrogenase containing FeMo-co. This set of hybrid enzymes provides a valuable platform for biophysical and enzymological studies aimed at unraveling key aspects of nitrogenase catalysis.

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F1.4

The endophytic role and growth-promoting ability of *Burkholderia alba* in *Rhizobium leucaenae*-common bean symbiosis

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Abstract

Rhizobium–legume symbiosis enables biological nitrogen (N2) fixation, representing a key strategy for sustainable agriculture. This process reduces reliance on synthetic fertilizers, which are associated with environmental concerns such as water, soil and atmosphere pollution. In addition to rhizobia, root nodules host a diverse community of endophytic bacteria that may enhance plant development through multiple plant growth–promoting (PGP) mechanisms. Despite the broad capacity of legumes to establish N2-fixing symbioses, common bean (*Phaseolus vulgaris*) is generally considered inefficient in biological N2 fixation compared to other grain legumes. The objective of this study was to improve common bean production by identifying new efficient endosymbiotic bacteria.

Using the trap-plant method, two interesting strains were isolated from nodules of common bean cultivated in soil samples collected from MBG-CSIC. Partial taxonomic identification based on 16S rRNA and housekeeping (*recA, glnII* and *atpD*) genes identified *Rhizobium leucaenae* as the endosymbiont. Interestingly, we also isolated an endophyte from the nodules that was identified as *Burkolderia alba* based on the sequence of its genome. The symbiotic efficiency of these new isolates was evaluated by inoculating seeds of common bean with *R. leucaenae* alone, or in co-inoculation with *B. alba*. Plant, nodule biomass and nitrogen content was higher in plants inoculated with the consortium *R. leucaenae-B. alba*. Interestingly, inoculated plants showed higher nitrogen content than KNO3 fertilized controls. Additionally, the PGP potential of *B. alba* was demonstrated through the evaluation of indole-3-acetic acid production, ACC deaminase activity, siderophores and biofilm production, and some nutrients solubilization. These results suggest that *B. alba* acts as a beneficial endophyte of *R. leucaenae*-common bean symbiosis. Consequently, this consortium is proposed as a promising biofertiliser for common bean that could help to

reduce the use of synthetic nitrogen fertilizers.

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F1.5

A global meta-analysis of soil fungal responses to changing fire regimes in ectomycorrhizal-dominated forests

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Abstract

Wildfire regimes are changing and intensifying globally [1], reshaping forest ecosystems and the belowground communities that sustain them. Ectomycorrhizal (ECM) fungi form critical mutualistic associations with dominant tree lineages across the Northern and Southern Hemispheres [2], regulating nutrient uptake and plant establishment [3], and thereby playing a central role in forest recovery after disturbance. However, global patterns of ECM and broader soil fungal responses to fire remain unclear and highly context dependent. Here, we present the first global meta-analysis synthesizing fire-driven changes in soil fungal communities across ECM-dominated forests. We compiled data from 64 peer-reviewed studies (>300 effect sizes), encompassing fungal richness, ECM root colonization, sporocarp abundance, and community composition. Using mixed-effects models, we assessed how fire severity, fire type (wildfire vs. prescribed fire), soil depth, biome (e.g.: Temperate, Mediterranean, Boreal…), and time since fire shape fungal responses. Across this methodological diversity, clear patterns emerged: fire did not uniformly reduce fungal diversity. Overall fungal richness and beta diversity showed no consistent global response, underscoring strong context dependency. However, this apparent resilience masked pronounced guild-specific declines. Both ECM and saprotrophic fungi exhibited significant declines in richness, alongside declines in ECM colonization and sporocarp abundance, indicating high sensitivity to fire of key functional groups. Fire type further modulated these responses. Wildfires, but not prescribed fires, drove substantial shifts in overall fungal richness and ECM community structure, suggesting that high-severity disturbances promote deeper compositional reorganization. Soil depth and time since fire emerged as critical modulators, revealing contrasting recovery trajectories across soil layers and biomes. Together, our results challenge the assumption of uniform fungal resilience to fire and highlight the importance of considering functional groups, fire characteristics, and belowground heterogeneity. As fire regimes continue to intensify, understanding these dynamics is essential to predict post-fire recovery and the stability of plant–fungal symbioses under global change.

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F1.6

Unlocking the genetic basis of soybean-rhizobia compatibility through GWAS in wild populations

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Abstract

Legume crops, particularly soybean (Glycine max), play an important role in sustainable agriculture due to their ability to form symbiotic associations with nitrogen-fixing rhizobia. This interaction results in root nodules where atmospheric nitrogen (N_2) is converted into ammonia, reducing the need for synthetic fertilizers and improving soil fertility in crop rotations [1]. However, this symbiosis depends on a highly specific molecular dialogue between host and rhizobia, which has been strongly shaped by domestication.

In this study, we focus on Glycine soja, the wild progenitor of cultivated soybean, which represents a valuable reservoir of genetic diversity often lost during the domestication of G. max. During this process, plants were selected for yield-related traits, including more efficient nitrogen fixation. This selection likely increased compatibility with rhizobia through the fixation of key symbiotic genes and the loss of genetic factors that restrict this interaction. [2]. In contrast, G. soja is often less compatible with rhizobia and frequently shows a binary nodulation phenotype. This suggests that wild genomes may have loci that restrict bacterial infection, making it a useful system to study the genetic factors that control rhizobial compatibility.

To dissect the multilocus genetic architecture underlying rhizobial compatibility, we implemented a Genome-Wide Association Study (GWAS) using a diverse panel of wild soybean accessions collected across multiple geographic regions in Asia [3]. A key component of this work was the design of the study population to maximize genetic diversity and statistical power. We conducted population structure analyses, including phylogenetic reconstruction and genetic distance estimation, to define subpopulations and guide accession selection.

Following genomic DNA extraction and high-throughput genotyping, our aim is

to identify loci and alleles associated with variation in nodulation and rhizobial compatibility. These findings will improve our understanding of plant–microbe interactions and support breeding strategies to enhance biological nitrogen fixation in soybean.

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SESSION 2

Molecular Biology and Physiology of Plant–Microorganism Interactions (I).

ORAL PRESENTATIONS

O2.1

Peptidoglycan remodelling in *Pseudomonas* modulate host perception, defense activation and root hair development

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Abstract

Plant–microorganism interactions play a key role in plant growth and health, yet many of the underlying molecular mechanisms remain poorly understood. The *yafL* gene, encoding an NlpC/P60 hydrolase involved in peptidoglycan remodelling, is enriched in plant-associated *Pseudomonas* spp. It is not only upregulated during plant–bacteria interactions, but also more abundant in rhizosphere microbiomes than in bulk soils1. A Δ*yafL* mutant has been shown to reduce growth promotion in *Brassica napus* and to induce shorter root hairs compared to the wild-type strain upon inoculation1. These findings suggest that the gain of this gene in *Pseudomonas* spp. is associated with adaptative evolution to the plant environment.
Here, we aimed to unravel the molecular basis underlying this altered plant phenotype. To this end, we performed an RNA-seq analysis of *B. napus* roots inoculated with either the wild-type or the Δ*yafL* strain. Our results reveal a differential plant response, likely mediated by jasmonic acid signalling, together with a reduction in auxin-related pathways. In addition, the downregulation of extensins, Casparian strip-associated proteins, and other structural components, combined with increased reactive oxygen
species (ROS) production and metabolic activity, suggests a shift towards a defense oriented physiological state. Notably, several plant peptidoglycan (PGN) receptors were downregulated, suggesting reduced perception of bacterial PGN signals. Collectively, these changes might indicate that the plant reallocates resources towards defense at the expense of growth, resulting in shorter root hairs and restricted bacterial entry.
Furthermore, given that the *yafL* mutation alters peptidoglycan remodelling, bacterial phenotypes related to cell wall remodeling and motility were analysed, revealing altered motility and secretion processes in the mutant strain compared to the wild type.
In summary, our results indicate that *yafL* acquisition modifies bacterial structure during plant interaction, supporting a shift towards increased compatibility with the plant host, potentially reflecting a more mutualistic interaction.

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O2.2

m^6A RNA methylation enables plant growth responses to microbialvolatiles by regulating growth-defense balance

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Abstract

Microbial volatile compounds (VCs) promote plant growth while modulating defense responses, yet the molecular mechanisms that enable plants to integrate these signals remain poorly understood. Here, we show that N^6-methyladenosine (m^6A) RNA methylation is required for growth promotion by microbial volatiles in Arabidopsis.
Mutants impaired in m^6A deposition (*mta*, *vir1*and *fip37*) or recognition (*ect2/ect3*) exhibit a strongly reduced growth response to volatile compounds, demonstrating that a functional m^6A pathway is necessary for plant responsiveness. Notably, volatile exposure does not alter transcript or protein levels of core m^6A writer components, indicating that pre-existing m^6A machinery is utilized without transcriptional induction.
Transcriptomic analyses revealed a striking convergence between independent m^6A-writer mutants, which share a minimal set of differentially expressed genes enriched in defense-related functions. Remarkably, this gene set is also induced in wild-type plants exposed to microbial volatiles, although in a context associated with growth promotion rather than autoimmunity.These genes are not enriched among direct m^6A targets, suggesting indirect regulation.
Together, our results support a model in which m^6A-mediated RNA regulation enables plants to balance growth and defense outputs in response to microbial volatiles. Loss of m^6A function shifts this balance toward a defense-dominant state that limits growth plasticity.

Funding

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SESSION 2

Molecular Biology and Physiology of Plant–Microorganism Interactions (I).

FLASH PRESENTATIONS

F2.1

NIN connects Nod factor signalling and ethylene biosynthesis in *Medicago truncatula*

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Abstract

The establishment of nitrogen-fixing symbiosis in *Medicago truncatula* is governed by a complex regulatory network in which ethylene serves as a critical checkpoint balancing bacterial infection and nodule organogenesis [1]. Despite the recognized role of ethylene as a negative regulator, the molecular mechanisms linking Nod factor signaling to ethylene biosynthesis remain poorly understood. We previously characterized the families of ACC synthases (ACS) and ACC oxidases (ACO), the enzymes responsible for ethylene biosynthesia, in *M. truncatula* [2]. Expression profiling showed that two *ACS* genes, *MtACS2* and *MtACS6*, are rapidly induced post-inoculation in a Nod factor-dependent manner. Because single *Tnt1* insertion lines lacked a clear symbiotic phenotype, we generated an *Mtacs2*/*Mtacs6* double mutant. Under symbiotic conditions, double mutant plants exhibited increased shoot biomass and higher nitrogen fixation rates compared to wild-type plants. While infection thread formation and nodule biomass remained largely unchanged, the double mutant developed numerous contiguous nodule clusters. This clustering suggests that these specific *ACS* genes mediate the local inhibition of nodule organogenesis. Furthermore, the double mutant displayed reduced ACC content specifically within nodules, but not in other tissues, indicating a nodule-specific regulatory role for these enzymes. Using electrophoretic mobility shift and transactivation assays in *Nicotiana benthamiana*, we demonstrated that the Nodule Inception (NIN) transcription factor directly binds and transactivates the *MtACS2* promoter. This work establishes a direct molecular link between the Nod factor signaling cascade and ethylene biosynthesis via NIN.

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F2.2

Functional characterization of a RelA-associated toxin-antitoxin system in *Sinorhizobium meliloti*

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Abstract

The establishment of an efficient Rhizobium-legume symbiosis depends on a tightly regulated molecular dialogue between the partners and on the bacterium's capacity to withstand multiple stresses encountered in the rhizosphere and inside the plant. The stringent response (SR) is a regulatory mechanism that allows bacteria to adapt to stressful conditions and is mediated by the phosphorylated nucleotides (p)ppGpp, also known as alarmones [1]. In *Sinorhizobium meliloti* (Sm), intracellular levels of (p)ppGpp are controlled by the long bifunctional enzyme RelA, whose absence in strain Rm1021 leads to a nodulation-deficient phenotype in alfalfa [2]. Recently, we found that inactivation of *relA* in the highly competitive Sm strain GR4 does not prevent nodule formation on alfalfa, suggesting diversification of the SR regulatory system among these bacteria.

The *relA* operon of Sm GR4 contains two genes, *vapB1* and *vapC1*, which code for components of a putative type II toxin–antitoxin (TA) module of the VapBC superfamily. VapC toxins are endoribonucleases whose activity is blocked by protein-protein interactions with their cognate antitoxin VapB [3]. TA systems have been implicated in bacterial stress adaptation and host interactions, acting in some cases as effectors during the SR. Interestingly, Rm1021 VapC1 lacks 40 amino acids at its C-terminal end compared with GR4 VapC1, which could impair the activity of this TA system and any function potentially coordinated with the SR in Rm1021. In this study, we evaluated the toxicity of GR4 VapC1 as well as the *in vivo* protein-protein interactions between VapB1 and VapC1. In addition, insights into the physiological role of VapBC1 in the free-living and symbiotic lifestyles of Sm GR4 will be presented through phenotypic characterization of single and double *vapBC* mutants.

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F2.3

Engineering nitrogenase electron-transport component in *Saccharomyces cerevisiae*

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Abstract

Nitrogen fertilizers, although fundamental for agriculture production, pose a significant environmental risk due to production processes and downstream environmental pollution. For this reason, alternatives need to be explored. Our group is focused on modular engineering of the nitrogen-fixation machinery (nitrogenase) in plant crops, using other eukaryotes as intermediate models[1]. In this work, we focus on the nitrogenase electron delivery module, typically constituted by the PFOR (pyruvate: ferredoxin/flavodoxin oxidoreductase) NifJ and the flavoprotein NifF. These are responsible in many organisms for the delivery of electrons to NifH, the Fe-component of the nitrogenase, then responsible for electron delivery to the NifD/NifK catalytic core[2,3]. Our goal is to find NifJ and NifF systems (or alternatives) able to effectively deliver electrons to the nitrogenase in yeast mitochondria. To do so, we are developing a multidisciplinary pipeline to explore various electron-transport components *in silico, in vitro* and *in vivo* and test compatibility between proteins deriving from different organisms, with a “mix and match” approach that aims to select the best option in terms of nitrogenase functionality and yeast fitness. Preliminary work showed that NifF from *Klebsiella oxytoca* is efficiently expressed in yeast mitochondria while NifJ proved difficult to engineer, due to poor expression and possible metabolic effects on the yeast cells. For this reason, a library of NifJ proteins and alternatives electron delivery proteins is currently being constructed. Some alternatives have already been tested and evaluated both in terms of functional compatibility with NifF (*in silico* and *in vivo*) and of expression and solubility in yeast mitochondria. Moreover, metabolic effects of PFORs on yeast are currently being investigated, leading to the identification of a major growth deficient phenotype that needs to be addressed as part of the engineering of electron delivery components for nitrogenase in yeast and other eukaryotic systems.

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F2.4

Elemental and isotopic characterization of *Terfezia claveryi* Chatin reveals key nutritional traits, trophic lifestyle and water use of desert truffles

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Abstract

Terfezia claveryi Chatin is a Pezizomycete fungus well adapted to arid and semiarid climates[1]. This species produces prized fruiting bodies called desert truffles mainly in the Mediterranean region and Arabian Peninsula, which have ecological, cultural, economic, nutritional, and medicinal value[1]. *T. claveryi* can establish ectendomycorrhizal interactions with Cistaceae shrubs, a distinctive mycorrhizal type characterized by both inter- and intracellular colonization, uncommon in other taxa[2]. Mycorrhizal synthesis has been achieved under greenhouse and *in vitro* conditions, and its cultivation has reached fruiting body production in the field[1]. However, the extent of its saprotrophic ability and its dependency on the host for mycorrhizal nutrition remain unknown. Isotopic characterization is a valuable tool to investigate fungal trophic lifestyles, widely used to discriminate between ectomycorrhizal (ECM) and saprotrophic fungi[3]. Considering the unusual mycorrhizal type of *T. claveryi* and its ecological role, this study aimed to provide an ecophysiological, isotopic, and elemental characterization of a *T. claveryi* orchard in Murcia, Spain, over two years (2021–2023). During this period, soil mycelial abundance remained stable, and the host plant, *Helianthemum almeriense*, exhibited the highest photosynthetic activity in winter. Elemental analysis of fruiting bodies revealed carbon content similar to other truffles and, along with *Tuber aestivum*[4], the highest levels of P, K, Ca, S, and Mg reported among truffles. The leaf and truffle $\delta^{15}N$ data revealed a heavy dependence of *H. almeriense* on *T. claveryi* for nitrogen acquisition, whereas Quadratic Discriminant Analysis (QDA) of $\delta^{13}C/\delta^{15}N$ isotopes placed *T. claveryi* clearly within the ECM trophic niche with no overlap with saprotrophs. Furthermore, their $\delta^{18}O$ and $\delta^{2}H$ isotope water evaporation line highlights the critical role of precipitation during the preceding fall in fruit body formation. Overall, this study advances understanding of *T. claveryi* trophic mode and water use, providing relevant insights on its fruiting and agricultural management.

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F2.5

Studies of differentially expressed genes in the *Lotus japonicus – Mesorhizobium loti* symbiosis

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Abstract

Transcriptomic studies carried out in our research group led to the identification of several genes that were differentially expressed between nodulated and non-nodulated plants of the model legume *Lotus japonicus*[1,2]. These genes were either down-regulated or up-regulated in leaves of *L. japonicus* plants inoculated with *Mesorhizobium loti* and encoded plant-specific glutaredoxins (GRXs) and an ethylene response transcription factor (ERF), none of them previously related to symbiosis.

In this work we show the results obtained in the study of these proteins through the characterisation of homozygous mutant plants affected by the insertion of the endogenous LORE1 retrotransposon grown under different nutritional conditions. We observed that plants affected in the genes that encode these GRXs and ERF showed significant changes in nodulation parameters or nitrogen fixation capacity compared to wild-type plants when external nitrate was supplied to plants growing under symbiotic conditions, suggesting a key role of these proteins in the systemic regulation of nitrate-dependent inhibition of nodulation.

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F2.6

EveD: an Extracellular Vesicle-Secreted Effector in Rhizobia

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Abstract

The rhizobium-legume symbiosis constitutes one of the most intimate partnerships found in nature. Different molecular mechanisms are involved in the establishment of this interaction, including: Nod factors, surface polysaccharides and various secretion systems, such as the Type III Secretion System (T3SS)[1]. Among these mechanisms, the bacterial Extracellular Vesicles (EVs), also referred to as the Type 0 Secretion System (T0SS), are emerging as important mediators of interkingdom communication. These lipidic structures transport a wide range of molecules and participate in diverse biological processes, while maintaining an effective concentration of active compound, a phenomenon known as quantal secretion[2,3].

Sinorhizobium fredii HH103 is a nitrogen fixing bacterium able to nodulate a broad range of legumes, in which EVs contribute to the establishment of symbiosis with legumes[4]. In this work, we studied a novel effector protein secreted via EVs by this bacterium, EveD. This protein was initially identified *in silico* as a putative T3SS effector (T3E) due to its partial homology with TALE (Transcription Activation Like Effector) T3Es from *Xanthomonas* spp[5]. However, our results demonstrate that EveD does not follow the canonical T3SS pathway. Thus, the *eveD* expression is not regulated in the same manner as typical T3E genes and EveD secretion is independent of the T3SS, relying instead on EVs.

Functional analyses indicate that EveD localizes to both the nucleus and membrane of the plant cell in the soybean roots, its natural host, suggesting that EveD has a role in host modulation. Interestingly, competitiveness assays show that an *eveD* mutant strain is more competitive than the wildtype strain, indicating that EveD influences early stages of the symbiotic interaction.

These findings support the idea that EVs have a role as an alternative secretion system and highlight the implication of the T0SS in rhizobia-legume symbiosis.

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SESSION 3

Molecular Biology and Physiology of Plant–Microorganism Interactions (II).

ORAL PRESENTATIONS

O3.1

Unveiling new metalloproteins in *Medicago truncatula* nodules

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Abstract

Symbiotic nitrogen fixation carried out in legume root nodules relies in a steady supply of metals [1]. To satisfy nodule requirements, transporters must transfer metals to cytosolic shuttling proteins (metallochaperones) to be delivered to the final accepting metalloproteins. As “free” metals are toxic [2], this must be carried out through protein-protein interactions, *i.e.* as a “bucket-brigade”. Therefore, metalation relies in the compatibility of docking interfaces and not simply relative metal binding affinities. We hypothesize that by identifying new metallochaperones we can isolatenew downstream metalloproteins. As a proof-of-concept, by characterizing *Medicago truncatula* Cu^{+}-chaperone NCC1 and Fe^{2+}-chaperone ICHAP1, we have identified novel nodule metalloproteins [3,4].

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O3.2

Bioprospecting c-di-GMP activated exopolysaccharides in beneficial plant-interacting bacteria

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Abstract

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 signalling 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 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. The rewiring of bacterial c-di-GMP signalling can be used as a wise approach to unlock 'hidden' EPS from environmental plant-interacting strains. In a recent 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 5-10% of the strains were identified as promising candidates for the c-di-GMP-dependent overproduction of novel EPS [1].

This genetic approach has enabled us to uncover and characterized novel EPS in different plant-interacting bacterial strain, including Mixed-Linkage β-glucans in different rhizobia [2,3], a Galacto-Sphingan in *Sphingomonas sp*.[1] and a novel thermoreversible gelling polysaccharide produced by *Paraburkholderia phymatum*[4].

These results position c-di-GMP as a versatile and attractive tool for exploring the hidden diversity of bacterial EPS with ecological value.

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O3.3

Multifaceted regulation of legume nodulation by the rhizobial small RNA NfeR1

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Abstract

The nitrogen (N) status transduced through the NtrBC two-component system is a major signal guiding transition of rhizobia from a free-living soil lifestyle to an intracellular state within legume root nodules. The *trans*-acting small RNA NfeR1 (Nodule Formation Efficiency) has emerged as a novel N-responsive regulator orchestrating this shift (1, 2, 3). NtrC and the LysR-type symbiotic regulator LsrB bind distinct sites in the *nfeR1* promoter, functioning antagonistically as transcriptional repressor and activator, respectively. This regulatory logic drives peak NfeR1 accumulation under N starvation and during nodulation. NfeR1, in turn, feedback silences the *ntrBC* mRNA by base-pairing at the *ntrB* translation initiation region, thereby tuning the strength of NtrC-mediated autorepression of the system. This mixed (protein-RNA) NtrBC-NfeR1 double-negative feedback loop enables a controlled shutdown of NtrBC activity upon nodule entry, aligning bacterial N metabolism with the symbiotic demands (2).
Using MS2 affinity purification coupled with RNA sequencing, we identified numerous additional NfeR1 targets across pathways central to symbiotic success, including N metabolism, motility, osmotolerance, and cell cycle control (3). Through broad regulation of cell-cycle mRNAs, NfeR1 influences bacterial morphology and DNA replication, while its repression of *gdhA* underpins *nod* gene activation by limiting glutamine dehydrogenase-dependent N assimilation. This regulation is further fine-tuned by an unprecedented RNA-RNA feedback interaction between NfeR1 and the dual-function sRNA SmelC549.
Our findings position NfeR1 as a central hub within a complex RNA-based regulatory network that integrates the N status with diverse physiological pathways, optimizing *S. meliloti* symbiotic performance.

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SESSION 3

Molecular Biology and Physiology of Plant–Microorganism Interactions (II).

FLASH PRESENTATIONS

F3.1

Cyclic di-GMP Modulation of Quorum Sensing and Its Impact on Type VI Secretion System Function in *Sinorhizobium fredii*

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Abstract

Effective rhizobium-legume symbiosis depends on multiple molecular signaling pathways, integrating not only classical nodulation factors and surface polysaccharides but also diverse protein secretion systems. Among them, the Type VI Secretion System (T6SS) has emerged as a key player, due to its dual roles in interbacterial competition and interactions with eukaryotic hosts, though its contribution to symbiosis remains unclear **[1]**. Key regulatory messengers, including the main autoinducer of the quorum sensing (QS) systems, the *N*-acyl homoserine lactones (AHLs), and the second messenger cyclic di-GMP (c-di-GMP), modulate the transition between motility and biofilm formation, especially in the context of bacteria interacting with eukaryotes, including rhizobia. While c-di-GMP's impact on exopolysaccharide production in these organisms is well established, its influence on protein secretion systems, particularly in conjunction with QS, is largely unexplored **[2]**. To contribute to the study of such interplay, we artificially increased intracellular c-di-GMP levels by overexpressing a heterologous diguanylate cyclase in three *Sinorhizobium fredii* strains of agronomic relevance **[3]**. This engineering revealed strain-specific outcomes, since elevated c-di-GMP enhanced biofilm development in two strains, but reduced it in another. Furthermore, using β-galactosidase expression assays, we confirmed that both high c-di-GMP and/or AHL concentrations contribute to the transcriptional activation of T6SS. These results demonstrate a direct regulatory link between c-di-GMP, QS signals, and T6SS expression, shedding light on the multilayered control mechanisms that structure beneficial rhizobia-plant interactions.

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Aranda-Pérez, Juan was awarded by a research grant (INV-PRE-2025-I-024) funded by MICIU/AEI/10.13039/501100011033. The attendance and presentation of this work by Aranda-Pérez, Juan was funded by MICIU/AEI /10.13039/501100011033 and FEDER, UE (proyect PID2024-159060OA-I00).

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F3.2

Microbial and light signalling converge to shape root system architecture

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Abstract

Plant–microorganism interactions can profoundly shape root system architecture through the integration of hormonal and metabolic, ultimately impacting plant fitness [1]. Emerging evidence indicates that beneficial bacteria can influence light signalling, pointing to a previously underexplored connection between microbial cues and photomorphogenesis [2–4]. However, how these processes are coordinated during plant–bacteria interactions remains poorly understood.

Here, we investigate how interaction with *Pseudomonas sp*. CDVBN10B modulates root architecture in *Brassica napus* under conditions that differentially restrict light perception in shoots and roots. Our results reveal that the reduction in primary root length is independent of light perception and primarily driven by bacterial auxin production, indicating a direct microbial control over root elongation. In contrast, both shoot growth promotion and lateral root development triggered by bacterial inoculation are dependent on light perception. This differential response indicates an uncoupling of root growth processes, with distinct outputs either dependent on or independent of light cues. These findings indicate that the bacterial effects on plant growth are tightly coupled to shoot light perception, likely reflecting the requirement for photoassimilates to sustain both aerial and root developmental programs. To further dissect this relationship, we employed reporter lines in *Arabidopsis thaliana*, to investigate how the presence of the bacteria affects the redistribution of carbon resources towards the root system. Together, our results indicates that bacterial colonization actively reprograms source–sink dynamics in a light-dependent manner. This work establishes a conceptual framework in which microbial signals and photomorphogenic cues converge to regulate plant developmental plasticity, providing new insight into how plants optimize growth in complex biotic and environmental contexts.

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F3.3

Engineering bacterial extracellular vesicles as tools for intracellular effector delivery and activation of the plant immune system

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Abstract

Bacterial extracellular vesicles (bEVs) are lipid-based and cell-detached nanospheres that have emerged as pivotal mediators in inter-kingdom communication, acting as nano-sized conveyors of proteins, lipids, metabolites and nucleic acids over long distances [1]. The application of bEVs as biotechnological tools in agriculture remains poorly explored, partly due to the challenge of proving effective cargo internalization into the host cytoplasm.

Firstly, our work sought to provide evidence of intracellular cargo release in *Arabidopsis thaliana* suspension cells. For this purpose, bEVs were labelled with a non-fluorescent dye that becomes fluorescent only upon entry into living host cells and following interaction with intracellular cytoplasmic proteins. Confocal microscopy revealed the presence of intracellular fluorescence within plant cells, proving membrane fusion and the functional delivery of the cargo into the host cytoplasm. To harness this delivery mechanism, we developed a "molecular vaccine" strategy by engineering *Pseudomonas putida* KT2440 bEVs. We utilized a scaffolding protein, JBO2, which is an outer membrane-anchored lipoprotein that fosters vesicle secretion to specifically load vesicles with the effector AvrRpt2 from the plant pathogen *Pseudomonas syringae* pv. tomato DC3000, that generates Effector-Triggered Immunity in *A. thaliana*. Thus, engineered bEVs triggered a marked Hypersensitive Response with localized necrosis [2]. This immune activation was further validated by the upregulation of the systemic defense marker gene *PR1*. Importantly, plants pretreated with engineered bEVs exhibited a significant reduction in subsequent bacterial pathogen proliferation, demonstrating effective biological protection. This work establishes an organism-free platform for plant priming, offering a sustainable alternative to traditional agrochemicals for enhancing crop resilience against pests.

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F3.4

The *Bradyrhizobium diazoefficiens* transcriptome in response to copper starvation under denitrifying conditions: Insights into the FixLJ-FixK_2 and RegSR-NifA regulatory cross-talk

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Abstract

The efficiency of biological nitrogen fixation in the soybean endosymbiont *Bradyrhizobium diazoefficiens* is intrinsically linked to its capacity to induce the high-affinity terminal oxidase *cbb*$_3$ in the bacteroids. Furthermore, *B. diazoefficiens* is able to denitrify under microoxic conditions in the presence of nitrate, in both free-living and symbiotic states. This process serves as a vital alternative respiratory pathway, although it also triggers the release of nitric oxide and nitrous oxide, two gases involved in climate change. Understanding how the cell balances oxygen (O_2) and nitrate respiration is crucial, as both processes are integrated into the master regulatory network composed of the FixLJ-FixK_2-NnrR and RegSR-NifA cascades, that responds to O_2 and nitrogen oxides [1]. Copper (Cu) has recently emerged as a critical modulator of denitrification genes [2]. However, the systemic impact of Cu availability on the *B. diazoefficiens* transcriptome under denitrifying conditions remains to be elucidated.

In this study, a RNAseq transcriptomic analysis of cells cultured under Cu-limiting denitrifying conditions revealed 986 differentially expressed genes compared to cells cultured under Cu-standard conditions. Among them, we identified genes involved in Cu homeostasis and transport, oxidative stress, and redox metabolism, such as the *fixNOQP* operon encoding the *cbb*$_3$ oxidase, as well as the *nirK*, *norC* and *nosZ* denitrification genes. Of the regulatory genes, only *regR* expression was significantly induced, suggesting a role for the RegR protein in the Cu-limitation response. Analysis of the expression of *fixK*$_2$, and a selected repertoire of its targets, including *fixNOQP* and structural denitrification genes, in a *regR* mutant background indicates that RegR is a repressor of FixK_2-mediated control. This inference was validated using EMSA assays, showing that RegR can bind to the *fixK*$_2$ promoter region. These findings reveal a new connection between the FixLJ-FixK_2-NnrR and RegSR-NifA cascades, whereby RegR controls the expression of denitrification genes via FixK_2.

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F3.5

Cultivar-specific flavonoid responses to arbuscular mycorrhizal inoculation under projected climate change scenarios

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Abstract

The progressive increase in atmospheric CO_2, temperature and drought affect grape composition. Our objective was to evaluate whether the association of two grapevine varieties, Tempranillo (TEM) and Cabernet Sauvignon (CS), with arbuscular mycorrhizal fungi (AMF) can mitigate the effects of climate change on grape ripening and flavonoid profile, using biochemical and molecular approaches. Plants of TEM and CS grafted onto R110 rootstock were inoculated with a consortium of five AMF and four PGPRs (+M) or with only PGPRs (-M). The plants were grown in greenhouses under two ambient conditions: current CO_2 concentration and ambient temperature (ACAT), or elevated CO_2 and temperature (700 ppm and ambient + 4˚ C, ECET). At veraison, two water availability treatments were applied, full irrigation (WW) or cyclic drought (D). For each variety, ambient conditions, water availabilities and inoculation treatments were arranged in a factorial design (2x2x2x2). In +M vines of both varieties, droughted plants showed lower total soluble solids during ripening under ACAT, whereas this effect was not so evident in -M. ECET reduced must acidity, in general when combined with D. In terms of flavonoid composition, TEM showed lower sensitivity to environmental conditions and AMF inoculation than CS. In CS, AMF reduced anthocyanin and flavonol concentrations at harvest when plants grew under ACAT and WW conditions. Regarding the relative effect of environmental stressors, -M CS plants experienced significant anthocyanin degradation under ECET applied alone or combined with D. However, AMF partially mitigated these effects and increased flavonol levels in this variety. The transcriptomic response to D was attenuated in +M plants compared to -M in both varieties, as evidenced by a reduced number of differentially expressed genes. The effect of environmental conditions on flavonoid composition in both -M and +M plants will be discussed in light of the RNAseq analyses.

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F3.6

FeMo-cofactor synthesis by a thermophilic nitrogenase lacking the NifEN scaffold

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Abstract

The biosynthesis of the Mo-dependent nitrogenase active-site cofactor (FeMo-co; Fe_7S_9C-Mo-homocitrate) begins with the formation of the Fe-S cluster precursor NifBco ($[Fe_8S_9C]$), synthesized by the NifB protein. This precursor is typically matured on the NifEN scaffold complex.[1] However, certain putative diazotrophic bacteria, such as the thermophile *Roseiflexus* sp. RS-1, lack *nifE* and *nifN*, suggesting an alternative FeMo-co maturation pathway.[2] In this study, *Roseiflexus* NifH, NifB, and apo-NifDK were heterologously expressed in *Escherichia coli*, purified under anoxic conditions, and biochemically characterized. $NifB^{RS}$ and $NifH^{RS}$ were independently validated as functional *in vitro*. Additional *in vivo* experiments in the diazotrophs *Klebsiella oxytoca* and *Azotobacter vinelandii* confirmed $NifB^{RS}$ and $NifH^{RS}$ carried out their expected functions. $NifDK^{RS}$ produced in *E. coli* was an apo form that contained P-clusters but lacked FeMo-co. Upon *in vitro* incubation with $NifB^{RS}$, $NifH^{RS}$, and substrates homocitrate, molybdate, and *S*-adenosylmethionine, at 48°C, apo-$NifDK^{RS}$ was reconstituted into an active Mo-nitrogenase, demonstrating that these three components are suKicient for full FeMo-co synthesis.[3] The reconstituted $NifDK^{RS}$ catalyzed N_2, proton, and acetylene reduction at ratios comparable to the *A. vinelandii* NifDK. These findings indicate that *Roseiflexus* NifDK functions both as the catalytic component and as cofactor maturase, representing a simplified, possibly ancestral pathway for FeMo-co biosynthesis that bypasses the canonical NifEN scaffold.

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SESSION 4

Agronomic and Ecological Uses of Plant–Microorganism Interactions.

ORAL PRESENTATIONS

O4.1

Evaluation of Social PGPR SynComs on Common Greenhouse Crops under Climatic Stress

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Abstract

The use of synthetic microbial communities (SynComs) as biofertilizers has gained interest due to the enhanced function and stability provided by increased microbial diversity. However, higher diversity leads to the emergence of new interactions between community members, which could affect the functionality of the consortium. The BSocial tool (http://m4m.ugr.es/BSocial.html) [1] addresses this by assigning social behaviours (positive, negative, or neutral) to individual strains based on their impact on net population growth. The latter can be applied to select "social consortia," composed of strains with positive or neutral behaviours, with increased plant growth promoting traits such as the production of siderophores and indoleacetic acid [2]. Furthermore, social behaviours have been found to remain stable despite environmental changes, allowing for the selection of highly functional and resilient consortia that are resistant to environmental stresses [3]. These studies enabled the selection of two social consortia based on their high resilience and functionality: X22 (Bacillus subtilis & Ensifer medicae) and X93 (*Azospirillum brasilense*, *Bacillus subtilis, Bradyrhizobium valentinum & Ensifer medicae*). Both consortia were tested for phytotoxicity, physiological growth and antifungal properties on common greenhouse crops: cucumber, pepper and tomato. Among the most significant results were an increase in root elongation between 37-59% and an increase in plant biomass between 34-90% in cherry tomato plants under conditions of combined water and salinity stress (irrigation at 50% with 450 mM saline solution) in both consortia. Additionally, neither of the two consortia showed phytotoxic effects on cucumber, pepper or tomato seeds, whereas germination index increased by >11% in all 3 crops. Both consortia demonstrated high potential for promoting plant growth, but also for biocontrol against phytopathogens (antagonism assays), where X22 and X93 completely suppressed growth of *Botrytis cinerea* and reduced growth of *Fusarium oxysporum* by 66% and 16% (X22 and X93, respectively).

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O4.2

Use of arbuscular mycorrhizal fungi as biostimulants for carotenoid biofortification in tomato fruits under field conditions

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Abstract

Carotenoids are bioactive compounds with important health-promoting properties, including antioxidant, anti-inflammatory, and anti-aging capabilities. Thus, they contribute to reducing the risk of diseases such as cancer, and cardiovascular, eye, skin, or metabolic pathologies. Therefore, a carotenoid-rich diet is essential for improving human health [1]. In plants, carotenoids act as phytoprotectants against photooxidative stress and as pigments, imparting colours ranging from yellow to red [2]. Soil beneficial microorganisms, including arbuscular mycorrhizal (AM) fungi, are used in agriculture as biostimulants to promote plant growth and development, as well as to increase their tolerance/resistance to environmental stresses [3]. However, their effects on fruit quality are less studied. Tomato is the most important fleshy fruit vegetable worldwide, containing high levels of nutrients and bioactive compounds. In this work, we have assessed the impact of early inoculation, during the nursery stage, of tomato seedlings with the AM fungus *Rhizophagus irregularis* on carotenoid content in fruits under real agronomic production settings, and using a commercial tomato variety. We have shown that early inoculation of seedlings with AM fungi provides long-lasting plant benefits that impact fruit quality, increasing the content of the carotenoids lycopene and β-carotene, the main coloured carotenoids in tomato fruits, more than 30%. We also showed that this increase was associated to a transcriptional upregulation of key genes of their biosynthesis pathway. Therefore, our results show that AM fungi, commonly used as biostimulants in agriculture, can also be used as a sustainable strategy for carotenoid biofortification in tomato production systems, contributing to the production of healthy 'functional products'.

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O4.3

Synthetic Assemblies for Microbiome-Based Agriculture (SAMBA): a trait-based framework for predicting and designing microbial consortia

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Abstract

The development of effective microbial biotreatments is often limited by the difficulty of predicting functional outcomes from individual strains and their combinations. To address this, we present Synthetic Assemblies for Microbiome-Based Agriculture (SAMBA), a trait-based framework that integrates biochemical profiling and machine learning to explore the rational assembly of microbial consortia. A collection of 124 rhizosphere bacterial strains isolated from maize fields, with 83% taxonomically identified, was characterized across 16 plant growth-promoting traits under both standard and PEG-simulated stress conditions.

Plant inoculation assays were conducted to quantify strain-level effects on growth and biomass, generating a dataset linking biochemical traits with phenotypic outcomes. Using this dataset, an ElasticNet-based classifier was trained to predict beneficial strains, incorporating engineered features such as stress-response ratios and interaction terms derived from biochemical traits. Model performance under stress conditions showed moderate predictive capacity (LOOCV F1=0.667, recall=0.770, AUC=0.655), while predictive power under non-stress conditions remained limited. Model interpretability analysis identified trait ratios associated with osmoprotection and mineral metabolism, particularly glycine betaine/polyamines and silica/polyamines, as relevant contributors to predicted plant-beneficial effects.

Building on these predictions, we explored multiple strategies for synthetic community (SynCom) assembly, including model-based ranking, trait coverage, and diversity-informed selection. Validation results identified a top-performing SynCom composed of *Pseudomonas putida*, *Pseudomonas juntendi*, *Priestia aryabhattai*, and *Paenibacillus glycanilyticus*. Preliminary validation showed moderate agreement between predicted and observed performance (Spearman r=0.36), suggesting that distinct biochemical strategies may contribute to functional complementarity within microbial consortia. Thus, SAMBA provides a conceptual framework for linking microbial functional traits to community-level outcomes, supporting the rational design of synthetic microbiomes. While still under development, this approach highlights the potential of combining trait-based screening with predictive modeling to guide microbial community assembly across diverse agricultural contexts.

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SESSION 4

Agronomic and Ecological Uses of Plant–Microorganism Interactions.

FLASH PRESENTATIONS

F4.1

Evaluation of combined root exudates and rhizosphere microbiome sampling to elucidate rhizosphere traits.

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Abstract

The rhizosphere is defined as a resource exchange site. Plant-derived organic compounds released in the rhizosphere, through root exudates, nurture a distinct microbiota that enhances plant health and nutrition. Investigating these processes is essential for understanding plant adaptation to the environment and represents the next frontier for developing sustainable crops. However, sampling root exudates and rhizosphere soil is challenging due to its low throughput and the process's destructiveness. While exudation collection requires intact roots connected to the shoot, rhizosphere soil sampling often damages the plant.

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. The sampling differed in 1) the bulk soil removal (rinse or shaking) and 2) the rhizosphere soil harvest from roots (dip or vortex). Absolute quantification of bacterial (16S rRNA gene) and fungal (ITS) rhizosphere communities did not identify differences between sampling approaches. Metabarcoding microbiome composition showed a rhizosphere profile distinct from the unplanted soil across sampling approaches for bacteria and fungi. Furthermore, most of the rhizosphere taxa were recovered across all approaches: 349 bacterial taxa comprising 78% of rhizosphere reads and 24 fungal taxa accounting for 73% of rhizosphere reads. However, compared to unplanted soil, approaches including vortexing showed a more distinct rhizosphere bacterial profile. Total carbon exudation rates and non-targeted exudate profiles were similar and consistent across different approaches. Conversely, root biomass and morphology measurements revealed that the rinse approach retained a significantly higher proportion of roots.

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. Nevertheless, our

observations also imply that the focus of the belowground investigation should guide the optimal strategy for sampling rhizosphere traits.

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Escudero-Martinez C*, Browne E, Schwalm H, [...], Oburger E* (1/11). Evaluation of combined root exudates and rhizosphere microbiome sampling approaches to elucidate rhizosphere traits. Accepted on New Phytologist 17 of march 2026 currently in editorial production DOI: 10.1111/nph.71174. *Corresponding authors.

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F4.2

Metabolomic and genomic characterization of *Pseudomonas chlororaphis* strains for biofungicide applications

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Abstract

Pseudomonas chlororaphis-based biofungicides are emerging as promising candidates due to their ability to combine multiple mechanisms, including antifungal activity, plant growth promotion, and environmental adaptability. We hypothesized that diverse *P. chlororaphis* strains possess distinct yet complementary metabolic and genetic traits that underpin their effectiveness as broad-spectrum biofungicides. To test this, nine *Pseudomonas* strains isolated from different plant hosts were evaluated for antifungal activity against major phytopathogens. *In vitro* assays were combined with metabolomic profiling using electrospray ionization liquid chromatography-tandem mass spectrometry (ESI-LC-MS/MS) and genomic analysis to characterize secondary metabolites and biosynthetic gene clusters. Strains were also assessed for plant growth-promoting traits, including indole-3-acetic acid (IAA) production, hydrogen cyanide (HCN) emission, enzyme activities, and phosphate solubilization. All strains exhibited significant antifungal activity, supported by the consistent production of key metabolite classes such as phenazines, siderophores, quorum-sensing molecules, and biosurfactants. Genomic analysis and functional assays confirmed the production of IAA and HCN, along with extracellular enzymes relevant to pathogen suppression and nutrient cycling. These findings provide a foundation for the rational design of synthetic microbial consortia with enhanced biocontrol and plant growth-promoting capabilities.

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F4.3

Different strategies for synthetic community design drive distinct rhizoplane colonization and plant growth outcomes in the wheat rhizoplane

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Abstract

Microbial colonization of the rhizoplane plays a key role in plant–microbe interactions and microbiome assembly, often involving biofilm formation on the root surface. However, identifying the microbial traits that drive community establishment on the rhizoplane and plant growth promotion remains challenging. In this study, we combined genome-based functional profiling with the design of synthetic microbial communities (SynComs), constructed according to different ecological and functional criteria, to evaluate how bacterial traits influence rhizoplane colonization and plant performance in wheat.

A total of 76 rhizoplane-associated bacterial isolates were genome sequenced and screened for functional traits related to surface colonization and plant growth promotion. Trait identification was based on the presence of complete functional pathways rather than individual genes. From these, 51 strains were selected to assemble 10 SynComs. Nine SynComs were designed according to different functional and ecological criteria, including enrichment in colonization-related traits, plant growth-promoting potential, and membership within the core microbiome at different time points, while one SynCom consisted of allochthonous strains included as a non-adapted community for comparison.

The SynComs were evaluated in a phytotron experiment with wheat plants, where shoot length and dry weight were measured over time (7–30 days post inoculation). Rhizoplane samples were collected at each time point for DNA extraction to monitor community dynamics and the persistence of inoculated strains. Significant differences in plant growth parameters were observed between treatments at 30 days post inoculation, with SynComs designed according to colonization-related traits and core microbiome membership showing the strongest positive effects on plant growth.

Overall, our results highlight that SynCom design strategy and strain selection criteria are critical for rhizoplane community establishment and plant growth outcomes, providing a framework for the development of effective microbial consortia.

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F4.4

Algal extracts stimulate plant growth-promoting bacteria and enhance tomato growth through microbial biostimulation

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Abstract

Algal biomass is increasingly explored as a sustainable agricultural biostimulant, yet its indirect effects on soil microbiota remain poorly understood. This study evaluated the capacity of algal extracts to stimulate beneficial microbial functions and enhance crop performance through microbiome-mediated mechanisms. A total of 17 macro- and microalgal biomasses were biochemically characterized and tested for their effects on soil microbial communities, plant growth-promoting bacterial (PGPB) traits and tomato growth. Soil microcosm experiments revealed that algal supplementation promoted the proliferation of beneficial bacterial taxa while maintaining overall community diversity and stability. In particular, treatments enriched taxa commonly associated with plant growth promotion, including Bacillus and Pseudomonas, without disrupting overall microbial diversity patterns. Culturable bacterial isolates recovered from these microcosms were subsequently screened for key PGP traits under algal supplementation. Across 16 bacterial isolates, several algal extracts significantly enhanced microbial functions including biofilm formation, auxin production, siderophore secretion, ACC deaminase activity and osmoprotectant production. These responses were strongly strain- and extract-specific, indicating that algal-derived compounds can selectively stimulate microbial functional traits.
Among the tested extracts, Ulva rigida consistently promoted beneficial microbial responses, stimulating proline and siderophore production across multiple bacterial isolates and exhibiting the highest carotenoid content among the analyzed biomasses. These microbial biostimulation effects translated into improved plant performance in tomato trials, where algal treatments significantly increased plant biomass relative to untreated controls and enhanced tolerance to biotic stress conditions. These findings demonstrate that algal extracts can act not only as plant biostimulants but also as microbial functional enhancers, promoting beneficial rhizosphere activities that improve crop productivity.

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F4.5

Impact of PGPR Addition to Compost on Nutrient Use Efficiency by crops and Soil Microbiome Composition

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Abstract

Compost is widely applied as an organic soil amendment due to its multiple agronomic and environmental benefits, including improvements in soil structure, organic matter content, and long-term soil fertility [1]. However, compared with mineral fertilizers, compost often exhibits a relatively slow release of plant-available nutrients, which can delay nutrient uptake by crops [2]. The use of plant growth-promoting rhizobacteria (PGPR) with organic matter-degrading capabilities and with microbial plant biostimulant (MPB) activity may accelerate nutrient turnover from compost and crops performance both directly through microbial activity and indirectly through modifications of the soil microbiome [3, 4].

In this study, we evaluated whether the application of a microbial consortium with degradative and MPB traits could enhance nutrient availability from compost amendments. A microcosm experiment was conducted using two crops, tomato (*Solanum lycopersicum* L.) and broccoli (*Brassica oleracea* L. var. *italica*), under three treatments: control soil without amendment, compost amendment, and compost combined with a bacterial consortium. Soil and plant nutrient concentrations (N, P, and K), nutrient use efficiency, crop biomass production, and changes in the structure and predicted functionality of the soil microbiome were assessed.

The combined compost + PGPR treatment significantly increased plant biomass, plant NPK accumulation, and nutrient use efficiency compared with the other treatments. Microbial inoculation also promoted marked shifts in the soil microbiome, increasing the relative abundance of microbial groups associated with organic matter decomposition, nutrient cycling, and other plant-beneficial functions. In contrast, compost applied without microbial inoculation mainly increased nutrient concentrations in soil but did not result in comparable improvements in plant growth and nutrient uptake.

Overall, these results suggest that PGPR act not only through their direct metabolic activity but also by reshaping the soil microbiome towards communities with greater mineralisation potential, thereby accelerating nutrient turnover and improving the efficiency of compost-based fertilisation strategies.

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F4.6

Inter-domain plant-Archaea interactions: halophilic archaea as biofertilizers in saline soils?

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Abstract

Recent metagenomics studies have demonstrated the presence of Archaea in the rhizosphere of halophytes [1]. Despite lower abundance compared to Bacteria, Archaea play a preponderant role in the C and N cycles, particularly in poor environments [2]. These results have prompted scientist to inoculate plants cultivated under saline conditions with halophilic Archaea [3]. In this work, we have inoculated tomato plants cultivated in the presence of salt with two haloarchaea, namely, *Haladaptatus* sp. and *Natronomonas aquatica*. Besides, the halophilic bacterium *Halomonas radicis* was used for comparison, together with non-inoculated controls and the mix of all prokaryotes together. The plant growth-promoting activities of archaea have been explored; *Haladaptatus* sp. showed phosphate solubilization and production of siderophores and biofilms, whereas *Natronomonas aquatica* displayed phosphate solubilization. By its part, *Halomonas radicis* solubilized phosphate and potassium, produced siderophores, and formed dense aerobic biofilms. Regarding their effects on plants, our results showed increase of 23 and 21 % in the fresh weight of shoots and roots upon inoculation with *Natronomonas*, although not significant due to large variations. The positive effect was not maintained in the presence of salt, despite microorganisms diminished the soil electric conductivity. Gas exchange determinations indicated a better intrinsic efficiency in the use of water. On the other hand, the number of flowers was greater in plants cultivated in salt, particularly those inoculated with both Archaea and the mix of all microorganisms. In conclusion, *Natronomonas aquatica* enhanced tomato growth, particularly in the absence of salt, and only slightly in the presence of salt; moreover, inoculation with haloarchaea led to better water use efficiency and promoted flowering, both important issues form an agronomical point of view.

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SESSION 5

Effects of Biotic and Abiotic Stresses on Beneficial Plant–Microorganism Interactions.

ORAL PRESENTATIONS

O5.1

Black truffle mycelium growth and host specific antioxidant response under combined atmospheric and soil drought stress

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Abstract

The black truffle (*Tuber melanosporum* Vittad.) is the most prized edible fungus of the Mediterranean basin, forming obligate ectomycorrhizal symbiosis with *Quercus* spp. Truffle production is highly sensitive to water availability and, thus, increasingly threatened by climate change [1].

Understanding tree-host responses to drought in this symbiosis is particularly relevant given that *Quercus ilex* L. and *Quercus faginea* L., the two most widely used tree-hosts in truffle plantations, display markedly different physiological strategies under water deficit [2], and that mycorrhizal fungi may enhance host antioxidant defences under stress. With this aim, one-year-old black truffle ectomycorrhizal *Q. ilex* and *Q. faginea* seedlings were nursery-grown combining high/low atmospheric vapour pressure deficit (VPD), with soil irrigation/drought treatments. Extraradical truffle mycelial biomass was qPCR-quantified [3]. Foliar oxidative damage was assessed by lipid peroxidation (MDA), while superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), and ascorbate peroxidase (APOX) were determined as indicators of oaks antioxidant response.

Contrasted host-dependent responses to drought stress were observed. Truffle mycelium biomass was modulated by VPD in the case of *Q. ilex*, whereas soil water availability and its interaction with VPD prevailed for *Q. faginea*. MDA was higher and SOD activity lower in *Q. faginea* than *Q. ilex*, consistently with the greater vulnerability to oxidative damage of the former host. Antioxidant enzymatic response to drought stress was tree species-specific. However, CAT activity was higher under low VPD regardless of tree species.

Our results point to tree host-specific regulation of *T. melanosporum* mycelium growth and indicate a deeply divergent antioxidant response of both oak species under combined atmospheric and/or soil drought stress. These findings may have direct implications for the management of black truffle agroforestry systems under future climate change scenarios.

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O5.2

Stress-induced root exudates recruit beneficial microbes and enhance stress tolerance in tomato

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Abstract

Plants exposed to abiotic stress can actively reshape rhizosphere microbial communities by altering the chemical composition of root exudates. Here, we investigated how drought and salinity stress influence root exudation in tomato (*Solanum lycopersicum*) and how these chemical shifts affect microbial recruitment and plant performance. Metabolomic profiling of root exudates revealed clear stress-dependent changes across developmental stages. In total, 121 metabolites were detected at two months and 63 at three months, indicating a transition towards a more specialized stress-associated exudate profile. Among the metabolites most strongly associated with stress conditions were mannitol and proline, which increased under drought, and shikimate and *myo*-inositol, which were enriched under salinity. These compounds are known to function as osmoprotectants and microbial signalling molecules in the rhizosphere.

Exposure of soil microbial communities to stress-derived exudates resulted in marked shifts in community composition. Microbiome analyses revealed enrichment of bacterial taxa commonly associated with plant growth promotion and stress tolerance, including *Bacillus*, *Streptomyces*, *Rhizobium* and *Pseudomonas*. Functional predictions based on KEGG and PLaBAse databases indicated a significant increase in the representation of microbial traits linked to phytohormone production, biofilm formation, osmoprotection and siderophore synthesis. From these communities, 13 bacterial isolates were recovered and experimentally characterized, confirming their ability to produce auxins, form biofilms, and synthesize osmoprotectants in response to selected exudate metabolites. Based on these metabolite–function relationships, strains displaying complementary traits were selected to construct three synthetic microbial communities (SynComs) representing control, drought and salinity conditions. When applied to tomato plants, these SynComs improved shoot and root development under drought and salinity compared with non-inoculated controls, demonstrating the functional relevance of exudate-mediated microbial recruitment. These results show how stress-induced root exudates can guide the rational design of microbial consortia to improve plant resilience under abiotic stress.

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O5.3

Arbuscular mycorrhizal fungal communities show greater resilience to climate change under organic management, enhancing soil multifunctionality in Mediterranean vineyards

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Abstract

Vineyards are emblematic Mediterranean agroecosystems, yet their sustainability is increasingly threatened by intensive management and ongoing climate change[1]. In these systems, soils are typically shallow, poor in organic matter, and prone to degradation, making belowground processes and plant–microbial interactions critical for long-term ecosystem health[2]. Here, we investigated how experimental warming and rainfall reduction, under organic versus conventional vineyard management, influence arbuscular mycorrhizal fungi (AMF) communities and their contribution to soil multifunctionality. Organic management consistently enhanced AMF richness, whereas climate treatments alone did not affect alpha diversity. However, warming and drought modified the phylogenetic structure and composition of AMF communities, especially under conventional management, indicating environmental filtering toward closely related taxa. Specific genera responded differently: *Rhizoglomus* and *Entrophospora* were indicators of stress under conventional management, whereas *Diversispora* became more prevalent under organic conditions. Organic management also led to higher and more resilient soil fertility, enzyme activities, and multifunctionality, while strengthening the relationships between AMF diversity, soil processes, and vine nutrition. In contrast, conventional soils were more sensitive to climate stress, showing weaker links between AMF composition and soil functionality. Finally, we found that AMF community composition and functional traits, rather than total richness, were the main drivers of soil ecosystem functioning. Overall, our results demonstrate that organic farming fosters stable and functionally relevant AMF communities, enhancing soil

multifunctionality and vine performance under climate stress, and thus offers a promising strategy to buffer Mediterranean agroecosystems against global change.

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SESSION 5

Effects of Biotic and Abiotic Stresses on Beneficial Plant–Microorganism Interactions.

FLASH PRESENTATIONS

F5.1

Responses of non-target soil microbial communities to double-stranded RNA application

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Abstract

Spray-induced gene silencing (SIGS) is an emerging non-transgenic crop protection strategy based on the application of double-stranded RNA (dsRNA). However, its effects on non-target soil microbial communities remain poorly understood. To support environmental risk assessment of dsRNA-based formulations, we evaluated the responses of soil bacterial and fungal communities to three different dsRNA constructs applied either via foliar spraying or soil irrigation in tomato soil microcosms. Using Illumina amplicon sequencing of the bacterial 16S rRNA gene and fungal ITS region, we assessed changes in microbial alpha and beta diversity at 7, 14, and 21 days after dsRNA application. Bacterial communities exhibited transient reductions in richness and functional changes, but persistent compositional shifts, particularly under irrigation-based treatments, indicating low resistance but high resilience. In contrast, fungal communities showed stable richness and function, but delayed compositional changes, suggesting greater resistance and slower responses to disturbance than bacteria. Overall, our results indicate that dsRNA-based SIGS applications entail relatively minimal short-term risk to soil microbial communities under the condition tested.

Funding

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F5.2

Environmental Drivers of Fungal Community Diversity: A Comparative ITS-Based Study Across Desert and Temperate Habitats

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Abstract

This study evaluates fungal diversity through ITS amplicon sequencing using Illumina technology in samples from desert environments (Atacama and Sahara) and a temperate ecosystem (Salamanca, Spain). Amplicons were analyzed according to both sample type (soil, plant, root, and rhizosphere) and region of origin (Atacama, Sahara, and Salamanca), revealing distinct diversity patterns shaped by environmental conditions.

At the regional level, desert samples exhibited higher fungal diversity in the rhizosphere compared to soil. In contrast, the temperate site showed greater diversity in soil samples, suggesting diBerences in community structuring between extreme and moderate environments, probably influenced by plant recruiting eBect. Regarding sample type, plant and root samples from the Atacama Desert displayed higher diversity compared to those from the Sahara and Salamanca. Additionally, rhizosphere samples from Atacama harbored significantly higher diversity than those from the Sahara, whereas soil samples showed the highest diversity in the temperate zone.

Notably, members of the family *Pleosporaceae* were among the most prominent taxa in plant-associated samples. This family has been previously reported as potentially involved in beneficial plant–fungus interactions [1], suggesting a possible ecological role in plant adaptation to environmental stress. Comparison between molecular data and previously isolated fungi from desert samples revealed overlap at the genus level, supporting consistency between culture-dependent and culture-independent approaches.

ITS sequence data were processed, and differential abundance analysis was conducted to compare desert and non-desert fungal communities, showing a significant enrichment of the class *Lecanoromycetes* in plant samples, a group associated with nitrogen fixation that may contribute to plant fitness. Overall, these findings provide insight into how environmental conditions shape fungal diversity and distribution, highlighting the key role of the rhizosphere in extreme ecosystems and

contributing to a better understanding of microbial ecology across contrasting biomes.

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F5.3

Plant-associated bacteria with nematicidal potential: A sustainable biocontrol strategy

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Abstract

Root-knot nematodes (RKNs, *Meloidogyne* spp.) are a group of plant-endoparasitic nematodes with a negative impact on plant health and agricultural productivity. As sedentary obligate endoparasites, infective second-stage juvenile (J2) RKNs penetrate the roots, inducing the formation of specialized feeding structures known as galls. These structures, which drastically reduce the plant's capacity to absorb water and minerals, among other physiological alterations, thereby negatively affecting crop yields [1]. The gradual banishment of effective but contaminant chemical nematicides, combined with climate change expanding the geographical distribution of these nematodes, makes it necessary to search for sustainable alternatives [2]. In this context, biological control using beneficial rhizosphere microorganisms is a highly promising strategy for protecting crops and improving overall soil and plant health.

This study aimed to evaluate the nematicidal potential of plant growth-promoting bacteria (PGPB) isolated from the *Triticum aestivum* (wheat) rhizoplane, and their ability to enhance plant resistance against the nematode *Meloidogyne javanica,* which infects cereals, among other crops. From a collection of 300 bacterial isolates, including 76 genome-sequenced strains, an *in silico* screening was performed to identify genes associated with nematicidal and plant growth-promoting traits. This led to the selection of six candidate strains. Subsequently, the bacterial extracts of these strains were evaluated *in vitro* for their activity against *M. javanica*. Results revealed that three of these strains, belonging to the genera *Pseudomonas* and *Pantoea*, exhibited significant nematicidal potential. Their nematode biocontrol efficacy was further validated *in vitro* using the model plant *Arabidopsis thaliana*. Additionally, their potential as dual biocontrol agents were evaluated by testing their antagonistic activity against agronomically relevant phytopathogenic fungi. Altogether, these findings provide a solid foundation for the development of novel biological tools against this global endoparasite, paving the way for future biocontrol agents and contributing to the transition toward a more resilient and environmentally friendly agriculture.

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F5.4

Drivers of arbuscular mycorrhizal fungal communities in Mediterranean agroecosystems under global change

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Abstract

Understanding the drivers of arbuscular mycorrhizal fungal (AMF) communities in agroecosystems is essential to predict their response to global change. Using a large multivariate dataset from 51 olive orchards across five countries within the Soil Olive project, we disentangled the relative roles of soil, climate, contaminants, management (organic, traditional, and high-density), and spatial structure in shaping AMF communities.

We focused on spore-derived AMF communities characterized by metabarcoding, which eliciently capture broad taxonomic and phylogenetic diversity, and applied an integrative analytical framework combining PCA-based variable selection, spatial eigenvector mapping (PCNM), and variance partitioning.

Abundance-based analyses revealed that climate and soil were the main drivers of AMF composition, followed by spatial structure, while contaminants showed only a minor independent contribution (~1%). Aridity emerged as the dominant climatic factor. However, this picture changed markedly when using presence–absence data: the explained variance increased substantially, uncovering stronger environmental signals. Together with the pronounced elect of contaminants on alpha diversity, this result indicates that pollution primarily impacts rare taxa, with limited influence on dominant community structure. In other words, contaminants reshape the “hidden” fraction of diversity rather than the core composition.

On the other hand, climate modulated management elects: increasing aridity led to a convergence of AMF communities across management types, whereas under milder conditions, organic, traditional, and high-density systems harbored more distinct communities.

Conclusion: climate—particularly aridity—sets the rules of AMF community assembly, while contaminants act as selective filters on rare taxa. Ignoring the rare biosphere may therefore underestimate the ecological impact of agricultural pollution on soil microbial diversity.

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F5.5

A new way to help their colleagues against the pathogen: *Trichoderma hamatum* induces plant systemic defenses by releasing elicitors from the *Sclerotinia sclerotiorum* cell wall

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Abstract

Trichoderma is a widely studied fungal genus used as a biological control agent and agricultural biostimulant [1]. In this study, we have described a novel mechanism of action whereby *Trichoderma* acts similarly to macrophages and dendritic cells in the human immune system, presenting antigens to T lymphocytes. Using *Trichoderma hamatum* [2-5], broccoli (*Brassica oleracea* var. *italica*) as a model plant, and *Sclerotinia sclerotiorum* as a model pathogen [6], we have determined how *Trichoderma* is able to release elicitors from the pathogen's cell wall, which are recognized by the roots and lead to a systemic activation of defenses against future foliar attack by the same pathogen. Liquid fermentation of *T. hamatum* on *S. sclerotiorum* mycelium was performed. In the resulting fungal filtrates, chitinase and β-endoglucanase activities were quantified, along with the amounts of glucosamine and glucan oligomers produced. These filtrates were subsequently applied to the roots of broccoli plants, which were later foliar-infected with the pathogen. Lesions produced were measured and different systemic defensive responses were evaluated through hormonomics, glucosinolate profiling and non-targeted metabolomics. In fungal filtrates of *T. hamatum* cultured on *S. sclerotiorum*, chitinase and β-endoglucanase activity was determined. These filtrates also contained the highest amounts of glucosamine and glucan oligomers. When applied to broccoli plants, the filtrates triggered a systemic defense response that was effective against the pathogen. This response was mediated by the hormones jasmonic acid, isopentenyladenine and ethylene, leading to the accumulation of antifungal compounds in the leaves, including glucobrassicin, niacin and several fatty acids. This defensive induction was not observed with glucosamine oligomers. Therefore, *T. hamatum* releases glucan oligomers from the cell wall of *S. sclerotionum* which may act as potential elicitors of systemic plant defenses.

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F5.6

Halophilic bacteria from Iberian salt flats enhance crop tolerance to salinity stress

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Abstract

Soil salinization is a major constraint to agricultural productivity, affecting approximately 20% of irrigated land worldwide and causing yield reductions of up to 20-40% in major crops. This problem is particularly severe in Mediterranean agricultural regions, such as the Tagus basin in Portugal, where estuarine dynamics contribute to progressive soil salinity. In this study, halophilic and halotolerant bacteria were isolated from saline environments across the Iberian Peninsula to evaluate their potential as microbial biotreatments for improving crop tolerance to salt stress. A diverse collection of bacterial isolates was obtained from saline and gypsic soils and screened for plant growth-promoting and salt-stress relieving traits under high salinity conditions (2 M NaCl). Functional characterization revealed widespread expression of traits associated with plant resilience, including ACC deaminase activity, auxin production, biofilm formation, exopolysaccharide production, siderophore secretion, nutrient solubilization and antioxidant production. Comparative analyses enabled the selection of several candidate strains with strong performance across multiple functional assays. Among these, strain DE and strain EA consistently showed the highest functional potential and salt tolerance.
Greenhouse experiments demonstrated that inoculation with these strains significantly improved plant performance under salinity in both maize (Zea mays) and tomato (Solanum lycopersicum), increasing shoot and root growth, biomass accumulation and photosynthetic efficiency relative to non-inoculated controls. Metabolomic analyses further revealed the secretion of osmoprotectant and stress-mitigating metabolites by the candidate strains, while transcriptomic analysis of maize plants inoculated with strain DE showed differential expression of genes associated with stress tolerance pathways, antioxidant responses and hormonal regulation. These findings highlight the potential of halophilic bacteria from natural saline ecosystems as bioinoculants for improving crop resilience in salt-affected agricultural soils.

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POSTERS

SESSION 1

Biological Nitrogen Fixation. Diversity of beneficial microorganisms for plants.

P1.1

Genomic Identification and Characterization of *nodD* Regulators in *Nostoc punctiforme*

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Abstract

Nostoc punctiforme is a filamentous, diazotrophic cyanobacterium capable of establishing symbiotic relationships with a wide range of plant species [1,3]. Despite its relevance as a model for cyanobacterial–plant symbioses, the molecular mechanisms underlying its association with rice remain largely unknown. In well-characterized interactions, such as those between legumes and nitrogen-fixing rhizobia, the common symbiotic signalling pathway (CSSP) is activated. This pathway is triggered when rhizobia secrete Nod factors in response to flavonoids produced by compatible hosts. We provided the first evidence of Nod-like protein expression in *N. punctiforme* and demonstrated the activation of CSSP components in rice during symbiosis [2]. Our results also highlight the role of lipochitooligosaccharides (LCOs) synthesized by *N. punctiforme* as key symbiotic signals.

Based on these previous results, the present study aims to elucidate the genetic basis of this interaction by characterizing *N. punctiforme* genes orthologous to the *nodD* regulatory gene of *Rhizobium leguminosarum*, a key symbiotic determinant controlling the expression of Nod factor biosynthetic genes. Three homologs of *nodD1* were identified in *N. punctiforme*, and loss-of-function mutants (Δ*nodD1*, Δ*nodD2*, and Δ*nodD3*) were generated through homologous recombination. Symbiotic assays revealed markedly reduced root adherence in *ΔnodD2* and *ΔnodD3* strains. Although all mutants retained the ability to colonize rice root cells, *ΔnodD2* exhibited a significant decrease in colonization efficiency, suggesting a key role for this gene in the establishment of the symbiotic interaction. These results provide new insights into the genetic determinants involved in the *N. punctiforme*–rice symbiosis.

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P1.2

XANES-Based Identification of Nitrogenase FeMo-cofactor Intermediates in Proteins, Bacteria and Yeast Systems

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Abstract

The development of nitrogen-fixing crops represents a promising strategy to reduce dependance on costly and polluting nitrogen fertilizers. A major challenge in this effort is the detection and characterization of intermediates involved in the biosynthesis of the nitrogenase enzyme, particularly the oxygen-sensitive iron-molybdenum cofactor (FeMo-co). In this study, we evaluated *in situ* spectroscopic approaches to elaborate the iron details present in these intermediates using X-ray Absorption Near Edge Structure (XANES) spectroscopy. Samples consisted of: (I) pure nitrogenase protein(s) accumulating FeMo-co and some of its precursors, (II) *Azotobacter vinelandii* strain variants that accumulate large-quantities of nitrogenase *in vivo*, and (III) engineered *Saccharomyces cerevisiae* that accumulates NifB-cofactor (Buren et al., 2019). Principal Component Analysis (PCA) of the XANES spectra successfully distinguished between fully assembled nitrogenase, intermediate cofactor synthesis, and non-expressing conditions. Furthermore, engineered yeast strains producing NifB-cofactor were clearly differentiated from controls. Although nano X-ray fluorescence (nXRF) analysis was limited by sample heterogeneity, XANES proved effective in detecting nitrogenase intermediates *in vivo*. These findings validate a new approach for monitoring cofactor biosynthesis and support future applications in plant systems, advancing efforts toward engineering nitrogen-fixing crops.

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P1.3

Cross-talk between N_2-fixation and nitrate assimilation in *Bradyrhizobium diazoefficiens* bacteroids

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Abstract

Bradyrhizobium diazoefficiens is a nitrogen-fixing bacterium widely used as a soybean inoculant. Under free-living conditions, this rhizobacterium assimilates nitrate (NO_3^-) through its reduction to nitrite (NO_2^-) and subsequent reduction of NO_2^- to ammonium (NH_4^+) by the action of NasC and NirA enzymes, respectively [1]. The aim of this work was to explore the role of NO_3^- assimilation under symbiotic conditions. Soybean plants, grown in the presence or absence of KNO_3, were inoculated with *B. diazoefficiens* USDA 110 (WT) and a *nasC* mutant. Analyses of leghemoglobin (Lb) in the nodules and nitrate reductase (NR) activity in the bacteroids indicated that NasC does not play a relevant role in bacteroids. These results suggest that *nasC* expression might be repressed by the NH_4^+ produced from N_2-fixation. To check this hypothesis, we analysed *nasC* expression in the nodules of a *nifH* mutant that is incapable to reduce N_2 to NH_4^+. We found that expression of *nasC* and NR activity were induced in the bacteroids of *nifH* nodules compared to WT nodules, both in the presence of NO_3^-. Moreover, bacteroids of *nifH,* defective in NH_4^+ production in the absence of NO_3^-, were able to produce NH_4^+ when plants were grown in the presence of NO_3^-. Furthermore, in the absence of NO_3^-, *nifH* nodules were very small, while in the presence of NO_3^- their size was significantly higher. These positive effects were not observed in the nodules of plants inoculated with a *nifHnasC* double mutant. Moreover, plants inoculated with the *nasC* mutant and starved of nitrate for 18 days had higher biomass, N content, and Lb levels in the nodules than plants inoculated with the WT strain. Taken together, these results suggest that NO_3^- assimilation is induced in the bacteroids when nitrogen fixation is impaired and, on the opposite, N_2-fixation is improved when NO_3^- assimilation is blocked in the bacteroids. We propose the existence of a cross-talk between these two processes.

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P1.4

Biotechnological potential of plant and soil microbiome from the high Andean moorlands

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Abstract

We present a recently funded project where we aim to study the structure and function of the microbiome in soil and native plants from moorland ecosystems in Ecuadorian Andes. These lands have remained microbiologically unexplored until now. However, it is an ideal environment for discovering resistant and highly specialized bacteria, whose biotechnological potential could be very fruitful for various industrial sectors. This is because environmental conditions in moorlands are quite extreme: attitude of 4,500 meters above sea level, intense solar radiation, temperatures with sharp day-night fluctuations, nutrient-poor soils, daily freezing and thawing, drying winds and low oxygen levels. Our team has carried out the first sampling campaign in Cerro Igualata to study rhizosphere and endophytic bacteria from *Valeriana nivalis* (valeriana de los Andes) and *Lycopodium crassum*. Both are endemic species used by local indigenous communities for medicinal purposes. We hope to find microorganisms with activities of interest to transfer for market-ready applications, such as plant growth-promoting properties for biofertilizers, antibiotic and lytic activities, among others. To this end, omics tools will be employed to conduct an initial screening of activities, followed by the targeted isolation of cultivable microorganisms of interest. In addition, the project aims to raise public awareness about the protection of valuable ecosystems such as the moorlands, as they are considered “water factories”. Their proper functioning is essential for water cycle and, therefore, for water supply to small communities, towns and cities.

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P1.5

A phylogenomic reappraisal of the family *Rhizobiaceae*: Proposals for eight novel genera and 32 novel combinations

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Abstract

The family *Rhizobiaceae* (class *Alphaproteobacteria*) is a highly diverse group currently comprising at least 276 validly published or proposed species across 38 genera. Despite recent efforts to refine its classification, significant taxonomic anomalies and inconsistencies persist, particularly within the genus *Rhizobium* [1, 2]. This study aimed to revisit the taxonomy of the family *Rhizobiaceae* by integrating new genomic data and applying a rigorous phylogenomic framework to resolve existing classification conflicts. We generated whole-genome sequences for 12 *Rhizobium* type strains that previously lacked publicly available genomic data. These were analyzed alongside a total of 242 *Rhizobiaceae* type strains. Taxonomic reappraisal was conducted using established phylogenomic pipelines, with a primary focus on core-proteome average amino acid identity (cpAAI) comparisons to delineate generic and species boundaries [1].

Our findings clarify several taxonomic disputes: we identified *Rhizobium aegyptiacum* as a later heterotypic synonym of *R. aethiopicum* and provided evidence contradicting the recent synonymization of *R. azibense* and *R. gallicum*. To address paraphyletic genera and account for clearly distinguishable monophyletic lineages, we propose the establishment of eight new genera (*Allohoeflea* gen. nov., *Arminella* gen. nov., *Gillisella* gen. nov., *Limnomicrobium* gen. nov., *Martinezella* gen. nov., *Neohoeflea* gen. nov., *Parahoeflea* gen. nov., and *Velazquezella* gen. nov.) and 32 novel combinations. Furthermore, we identified the potential loss of the *Rhizobium arsenicireducens* type strain, necessitating the designation of a neotype.

This comprehensive reordination provides a more stable and phylogenetically accurate nomenclature for the *Rhizobiaceae*, facilitating future research on these greatly appreciated bacterial family.

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P1.6

Martinezella and *Paraburkholderia* are microsymbionts of *Mimosa foliolosa* and *Mimosa bimucronata* in Brazil

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Abstract

Mimosa is a legume from the tribe Mimosae whose species are common in several Brazil regions such as the Cerrado [1]. The species of this genus are able to establish symbiosis in Brazil mainly with beta rhizobia [2,3], whose diversity has been analysed in some locations at the Brazilian Cerrado [4]. Nevertheless, there are no data about the identity of strains nodulating Mimosa species in some protected areas with ecological relevance, such as the State Park of Itacolomi and the National Park of Serra do Cipó within the Brazilian Cerrado. Therefore, in this work we analysed several strains isolated from nodules of *Mimosa foliolosa* and *Mimosa bimucronata* growing in these two Parks. These strains were identified with MALDI-TOF MS, PCR-RAPD fingerprinting and 16S rRNA gene sequence analysis. According to the results obtained, the strains belong to the genera *Martinezella* (formerly *Rhizobium*) and *Paraburkholderia*. The *Martinezella* strains were closely related to the species *Martinezella lusitana*, *Martinezella rhizogenes* and *Martinezella hainanensis* (with similarity values ranging from 97% to near 100%). As for the strains belonging to Paraburkholderia, they were closely related to *Paraburkholderia guartelaensis* and *Paraburkhoderia atlantica* with with similarity values ranging from 98% to near 100%. These results showed that some of these strains belong to new species of both *Martinezella* and *Paraburkholderia*.

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P1.7

Pseudomonas retamae RB5^T, a non-rhizobial nodule endophyte with plant growth-promoting and stress-tolerance traits

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Abstract

Legume root nodules are primarily known as niches for rhizobial symbionts involved in biological nitrogen fixation, but they also harbor diverse non-rhizobial endophytes whose ecological roles remain insufficiently explored. Here, we report the genomic and functional characterization of strain RB5^T, isolated from root nodules of *Retama monosperma* growing in coastal dunes of northwestern Algeria and recently described as *Pseudomonas retamae* sp. nov. A polyphasic approach combining phylogenomics and genome-based species delimitation confirmed RB5^T as a distinct species within Pseudomonas. Beyond taxonomy, the strain displayed multiple plant growth-promoting traits, including ammonia and indole-3-acetic acid production, phosphate solubilization, motility, and antagonistic activity against *Fusarium oxysporum*. RB5^T also tolerated elevated salinity and temperature, supporting adaptation to harsh conditions typical of arid and semi-arid ecosystems. Greenhouse and field assays indicated significant improvements in wheat seedling vigor and biomass, including under drought-related stress. Whole-genome analysis further revealed genes associated with auxin-related pathways, phosphate acquisition, stress tolerance, antimicrobial potential, and nitrogen metabolism, consistent with a multifunctional plant-beneficial lifestyle. These findings highlight the overlooked contribution of non-rhizobial nodule endophytes to legume-associated microbiomes and support *Pseudomonas retamae* RB5^T as a promising candidate for sustainable agriculture. Future work will assess its role in nitrogen cycling and compatibility in microbial consortia with diazotrophic symbionts, with particular relevance for Mediterranean drylands under increasing climate variability and water limitation.

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POSTERS

SESSION 2

Molecular Biology and Physiology of Plant–Microorganism Interactions (I).

P2.1

Tracking mating-type dynamics of *Terfezia claveryi* in mycorrhizal plants and soil across nursery-field transition

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Abstract

Terfezia claveryi is a hypogeous fungus that forms desert truffles through ectendomycorrhizal symbiosis with Cistaceae plants in arid and semiarid environments[1]. *MAT* genes are the master loci controlling sexual reproduction and development in fungi[2]. *T. claveryi* is a haploid and heterothallic species as its strains harbor *TcMAT1-1* or *TcMAT1-2* idiomorphs, which include the *TcMAT1-1-1* and *TcMAT1-2-1* genes, respectively[3]. However, the spatio-temporal dynamics of these idiomorphs in plants produced in nursery and then transferred to field remain poorly understood. This research addresses this gap by examining the distribution patterns of the two *T. claveryi* mating-types in *Helianthemum almeriense* mycorrhizal plants and in surrounding soil, sampled before and after plant transplantations into open-field conditions over a 27-month period. *MAT*-specific primer pairs were designed and employed in a reproducible nested-PCR-based approach. In addition, the biomass of *T. claveryi* was quantified in all samples. Interestingly, a significant increase in *T. claveryi* mycelium was detected six weeks after transplantation in field. However, *T. claveryi* mycelium abundance was likely influenced by microenvironment heterogeneity and the adaptation of the symbiosis to the new conditions. According to the mating type distribution at nursery stage, both idiomorphs were found in root samples, with frequencies fluctuating over time. In contrast, the root-surrounding soil compartments exhibited a predominance of *TcMAT1-1-1*, which was consistently detected across all sampling points, indicating its possible early establishment. At field stage, this pattern became even more pronounced, as most of the samples were dominated by *TcMAT1-1-1*. Overall, *T. claveryi* soil colonization appears to result from a highly selective process with strains harboring *TcMAT1-1-1* idiomorph likely outcompeting those harboring *TcMAT1-2-1* idiomorph, at least under our experimental conditions. This research provides reliable markers to track the spatio-temporal distribution of strains of opposite mating types. In turn this is crucial to drive management practices to promote desert truffle spreading and fructification.

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P2.2

RNAseq studies reveal several *Sinorhizobium fredii* HH103 trans-sRNAs putatively involved in symbiosis with legumes

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Abstract

Sinorhizobium fredii HH103 is a broad-host range rhizobium capable of establishing a symbiotic relationship with many legumes. This relationship involves complex molecular communication, with various molecular signals from both parties. On the bacterial side, these signals include lipochitooligosaccharides (LCOs), also known as Nod Factors (NF), effector proteins secreted by the Type III secretion system (T3SS), and different surface polysaccharides. Previously, we have investigated the regulation of the production of these signals at the transcriptomic level [1-4], but the role of small RNAs has been overlooked. In addition, the genome sequencing of HH103 left different gaps in plasmid d, which is the one carrying most of the symbiotic bacterial genes. To address these issues, we have carried a new genome sequencing by using PacBio technology. Concurrently, we have employed a specialized RNA-seq technique called Cappable-Seq to identify transcription start sites (TSSs) in our bacteria, complemented by Term-Seq to identify transcription terminations with the final goal of characterise the repertoire of *trans*-sRNAs of HH103. By integrating this data with strand-specific transcriptomic analyses that were conducted using total RNA extracted from different treatments (free-living bacteria grown either in the absence or presence of flavonoids, bacteroids of different host legumes), we compared differently expressed TSSs in bacteroids from indeterminate (*Glycyrrhiza uralensis*) and determinate nodules (*Glycine max*). The aim was to identify *trans*-sRNAs potentially involved in the symbiotic relationship with these two kinds of legumes. We have identified six candidates showing enhanced expression in bacteroids of *G. max* and/or *G. uralensis*. Our current goal is to elucidate the putative symbiotic roles of these noncoding RNAs by deleting the regions that contain them and conducting nodulation assays with these legumes.

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P2.3

Engineering mitochondrial-targeted nitrogenase expression in *Saccharomyces cerevisiae*

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Abstract

Engineering eukaryotic cells capable of atmospheric nitrogen fixation represents a transformative strategy to reduce reliance on synthetic fertilizers, with major implications for sustainable agriculture and environmental protection [1]. A key challenge is the extreme oxygen sensitivity of nitrogenase, whose Fe–S clusters are rapidly inactivated under aerobic conditions. To mitigate this, we target nitrogenase to mitochondria, where respiratory activity contributes to maintaining low local O_2 concentrations [2,3]. We use *Saccharomyces cerevisiae* as an initial model system for nitrogenase engineering. To enable coordinated expression of the large gene set required for nitrogenase assembly while minimizing homologous recombination, we have developed a modular genomic integration platform. The system incorporates a diverse panel of promoters and terminators, focusing on galactose-inducible and hybrid regulatory elements that combine low-to-moderate basal expression with strong induction under controlled conditions. We quantitatively characterized these regulatory elements using GFP reporters, establishing a performance map of promoter strength, terminator efficiency, and expression tightness under non-induced versus induced conditions. In addition, comparison across five genomic integration loci revealed locus-dependent variation in expression levels, providing an additional layer for pathway optimization. Constructs are assembled via MoClo, integrated into defined genomic sites, and selection markers are excised using the Cre–Lox system. In total, more than 230 genetic parts (>40 promoters, >20 mitochondrial targeting signals, >130 *nif* and *nif*-related genes, 6 epitope tags, and >25 terminators) have been domesticated. Ongoing work focuses on protein purification and activity assays to optimize Fe–S cluster delivery and maturation in target Nif proteins.

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P2.4

HdmAB and FleN ae implicated in regulation of motility and biofilm formation in *Pseudomonas ogarae* F113 through protein/protein interactions

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Abstract

The AmrZ-FleQ hub controls rhizosphere adaption of *P. ogarae* F113, regulating among others, motility and biofilm formation in a c-diGMP dependent manner (Blanco-Romero et al., 2023). Both AmrZ and FleQ may interact with other proteins affecting c-diGMP concentration, motility and biofilm formation. The FleN protein can interact with FleQ in a 2 (FleN):4(FleQ) relation. We have modelled this interaction that appears to be favoured by ATP. We have confirmed this interaction by bacterial two-hybrid (BACTH) analysis. A *fleN* mutant shows reduced motility and reduced biofilm formation. This mutant also shows a reduction in c-diGMP production. Similarly, the AmrZ protein can interact with other proteins. Here we show that three HD-OD proteins can interact with AmrZ. Phenotypic analysis of mutants showed that the *sadB* mutant shows an increase in motility and a reduction in biofilm formation. Conversely, the *hdmA* and *hdmB* mutants are impaired in motility, but are not affected in biofilm formation. Analysis of the expression of the flagellin encoding gene, *fliC*, showed that *sadB* and *hdmA*, but not *hdmB* are implicated in the synthesis of the flagellar apparatus. We have modelled the interaction of SadB, HdmA and HdmB with Amrz. Interaction was predicted for the three pairs and confirmed by BACTH. The results presented here highlight the importance of protein/protein interactions in the regulation of motility and biofilm formation and in the adaption of *Pseudomonas* to the rhizosphere environment.

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P2.5

Exploring the Relationship Between *nifH* and *phaC* Genes to Enhance PHA Biosynthesis

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Abstract

The production of polyhydroxyalkanoates (PHA) by mixed microbial communities (MMCs) using agro-industrial waste represents a more environmentally friendly and sustainable biotechnological alternative for generating bioplastic precursors. Production of PHA by bacteria using an MMC is based on the development of an adequate enrichment of PHA-accumulating bacteria when nitrogen is limited in the presence of excess carbon. Among PHA-producing microorganisms, nitrogen-fixing bacteria are particularly notable for their ability to grow under nitrogen-limited conditions, thereby promoting higher PHA yields [1]. Nevertheless, the dynamics underlying the enrichment of these key groups during PHA accumulation have not been extensively explored. The main aim of this study was to evaluate the functionality and diversity of nitrogen-fixing bacteria within a PHA-accumulating MMC. To achieve this, a pilot-scale sequencing batch reactor (SBR) fed with used cooking oil as a carbon source and operated for 104 days under a feast/famine regime (an initial subjection of the bacterial culture to an excess of carbon source (feast), followed by a carbon deficiency (famine) under a nitrogen limitation). The diversity of the diazotrophic community was analysed through Illumina sequencing of the bacterial 16S rRNA gene. In addition, *nifH* and *phaC* gene abundances, determined by qPCR, were used as proxies for quantifying diazotrophs and PHA biosynthetic bacteria, respectively. PHA accumulation in biomass was determined by gas chromatography, obtaining values between 29.80 wt. % and 54.32 wt. %. Reactor operation resulted in the establishment of an MMC dominated by *Aquabacter*, a recently identified nitrogen-fixing genus. Moreover, a higher abundance of the *nifH* gene was associated with increased PHA-accumulating populations, as reflected in improved PHA yields. Overall, these findings highlight the pivotal role of the functional relationship between nitrogen-fixing and PHA-producing bacteria in enhancing PHA storage capacities and underscore the use of an enriched nitrogen-fixing community as a promising biotechnological alternative to conventional plastic production.

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P2.6

A host-dependent small heat shock protein contributes to *Rhizobium leguminosarum*/*Pisum sativum* symbiosis

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Abstract

The *Rhizobium*-legume symbiosis is the result of a molecular dialogue among both partners that leads to the formation of the root nodules in which bacteria are differentiated into bacteroids, the N_2-fixing form of rhizobia. During the establishment of the symbiosis, rhizobia are exposed to hostile microenvironments to which they must adapt to obtain an effective symbiosis. A proteomic analysis revealed the existence of proteins differentially expressed in bacteroids induced by *Rhizobium leguminosarum* bv. viciae *(Rlv*) UPM791 in pea and lentil nodules [1]. Among these proteins, host-specific stress-related proteins including small heat shock proteins (sHsp) were identified. sHsps are molecular chaperones that maintain protein homeostasis by preventing the irreversible aggregation of denatured proteins in response to different types of stress. One of them, RLV_1399, was observed to be expressed at high levels in pea bacteroids. In this work, it has been demonstrated that RLV_1399 is required for symbiotic performance of *Rlv* UPM791 with pea plants and to obtain maximum levels of nitrogen fixation in this host. Expression of *rlv_1399* gene was induced under microaerobic conditions in a FnrN-dependent manner consistent with the presence of an anaerobox in its regulatory region. Overexpression of RLV_1399 improves cell tolerance against oxidative stress and cationic nodule-specific cysteine-rich (NCR) antimicrobial peptides, two relevant stress factors present in the nodule. Co-purification experiments have identified a wide range of proteins as potential substrates for RLV_1399. Among others, proteomic analysis identified other sHsps and ATP-dependent chaperones suggesting that RLV_1399 participates in the protein quality system in the bacteroid. Interestingly, nitrogenase structural subunits were identified as major RLV_1399 interactors, suggesting that it is required to achieve optimal levels of nitrogen fixation in symbiosis with pea plants. Together, these results indicate that RLV_1399 is part of the bacterial stress response to face specific stress conditions offered by each legume host.

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P2.7

A *Sinorhizobium fredii* HH103 TetR transcriptional regulator putatively involved in symbiosis

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Abstract

Rhizobia are soil proteobacteria able to establish a symbiotic nitrogen-fixing interaction with legumes. Rhizobia infect legume roots and intracellularly colonize new plant organs called nodules. Within these nodules rhizobia differentiate into bacteroids able to fix N2 and provide combined nitrogen to the plant. The nodulation process is based on a complex molecular dialogue established between both partners that starts with the exudation of plant flavonoids that interact with the rhizobial transcriptional regulator NodD. *Sinorhizobium fredii* HH103 is a broad host-range rhizobial strain able to nodulate dozens of legumes including *Glycine max* (soybean). In this strain, NodD1 is the main global regulator controlling the production of symbiotic signals, but a series of additional transcriptional regulators such as MucR1, NodD2, NolR, SyrM, and TtsI have a role on this symbiotic regulon [1,2]. RNAseq studies revealed that SFHH103_03749, a gene coding for a TetR transcriptional regulator, showed slightly enhanced expression in symbiotic conditions [1,2,3]. This gene is located close, and in opposite direction, to a putative operon encoding an efflux pump. We have carried out qPCR studies and β-galactosidase assays showing that the product of SFHH103_03749 represses its own expression as well as that of SFHH103_03750 (coding for a component of the efflux pump). In addition, we have constructed mutants in both SFHH103_03749 and SFHH103_03750 and we will analyze its symbiotic phenotype with soybean, a HH103 host plant, Finally, we are carrying RNAseq studies to characterize the set of genes that are regulated by the product of SFHH103_03749.

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P2.8

Multi-omic analysis of the *Lotus japonicus* hemoglobin mutants *glb1-1* and *glb2-1*

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Abstract

Legumes contain two main types of hemoglobins: leghemoglobins (Lbs) and phytoglobins (Glbs). Lbs are found in the nodules, where they transport O_2 to the bacteroids, whereas Glbs are widely expressed in all plant organs. Glbs are divided into three classes according to phylogeny and biochemical properties. The *Lotus japonicus* genome encodes three Lbs and six Glbs. Previous research has shown that class 1 Glbs act as nitric oxide scavengers during the initial stages of rhizobial infection [1]. Glb2-1 is phylogenetically related to Lb but show intermediate properties between Lbs and class 2 Glbs [2].

To gain insight into the functions of Glb1-1 and Glb2-1, single knockout mutants from the *LORE1* collection were used. The *glb1-1/2-1* double mutant was obtained by crossing and homozygous lines were selected. The phenotype of seven-week-old nodulated and nodulated plants was examined. All three mutants showed delayed growth which could be caused by metabolic and/or hormonal alterations. To investigate this, primary metabolism and the hormonal and protein profiles were analyzed. We observed accumulation of asparagine and glutamine in the leaves of both single mutants, indicating alterations in nitrogen assimilation, as well as accumulation of pipecolic acid in *glb1-1*. As for the hormones, we detected a significant increase in salicylic acid in *glb1-1* and jasmonic acid in *glb2-1*. In the proteomic analysis, we found accumulation of a pathogenesis-related class 10 protein in *glb1-1*. Overall, our results strongly suggest that the absence of Glb1-1 and/or Glb2-1 induces stress in the plant, leading to changes in hormone contents which depend on the mutant, the plant organ, and the nitrogen source. Experiments to investigate the link between Glb1-1, Glb2-1, and hormone signaling (specifically salicylic acid and jasmonic acid) are underway. These experiments involve analyzing the expression profiles of the key genes participating in the biosynthesis pathways of the two hormones.

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P2.9

Structural studies of leghemoglobins and phytoglobins of the model legume *Lotus japonicus*

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Abstract

Leguminous plants have a wide variety of hemoglobins with distinct structures and functions. The genome of *Lotus japonicus* (Lotus) contains nine hemoglobin genes encoding three leghemoglobins (Lbs) and six 'nonsymbiotic' or phytoglobins (Glbs). The chemistry of hemoglobins is dictated by the heme group and its surrounding environment. In typical pentacoordinate (5c) hemoglobins, such as Lbs, a "proximal" His coordinates the 5th position of the Fe, leaving the 6th position open for reversible O_2 binding. In hexacoordinate (6c) hemoglobins, such as some Glbs, the 6th position is usually coordinated to a "distal" His. Previous studies, mainly on soybean Lbs, indicated that Lbs are 5c both in the ferric (3+) and ferrous (2+) states [1]. We have partially purified native Lbs from Lotus nodules and obtained their UV-visible spectra. Surprisingly, these Lbs are 6c in the 3+ state and 5c in the 2+ form, and therefore they are still able to transport O_2. We are now comparing soybean and Lotus Lbs by structural modeling to find out the reason for their difference in heme coordination when the proteins are in the 3+ form. In addition, we are focusing our studies on hemoglobin Glb3-1, which is also highly expressed in Lotus nodules and is important for symbiosis. Legumes contain Glb3-1 whereas all plants, including legumes, contain another class 3 Glb that is expressed in all tissues. Thus, the single Glb3 of Arabidopsis is equivalent to Glb3-2 of legumes rather than to Glb3-1 [2]. In this context, our aim is to characterize the differences between Glb3-1 and Glb3-2 of legumes by modeling their 3D structures and by attempting to crystallize Glb3-1 under at least 700 distinct conditions. Finally, an in-depth study is being carried out with recombinant Lotus Lb2 and Lb3 using site-directed mutagenesis of specific amino acids within the heme cavity; in particular, Tyr30Phe, His61Val, and the double mutant Tyr30Phe/His61Val.

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P2.10

A predictive model to identify targets for microoxic-responsive transcription factors in rhizobia

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Abstract

Rhizobia establish nitrogen-fixing symbiosis with leguminous plants under oxygen-limiting conditions (microoxia). The perception and transduction of the microoxia signal in rhizobia is mediated by conserved proteins, that are integrated into specific regulatory networks [1]. In *Bradyrhizobium diazoefficiens*, the soybean endosymbiont, the $FixK_2$ transcription factor plays a central role in the FixLJ-$FixK_2$ regulatory cascade, which perceives microoxia at the level of the FixLJ system [2]. In *Rhizobium leguminosarum*, which establishes symbiotic relationships with vetch, pea, lentil or clover, microoxia perception occurs at two levels, through the alternative hFixL-FxkR-FixK cascade and via the FnrN regulator, an orthologue of $FixK_2$ [1].

We propose a novel multidisciplinary strategy to predict and validate targets of microoxic-responsive transcription factors in rhizobia taking as working models $FixK_2$ from *B. diazoefficiens* and FnrN from *R. leguminosarum* bv. *viciae*. Using the experimentally validated $FixK_2$ consensus motif derived from the promoter regions of its known targets [3], we developed a sequence motif-based computational model that can scan promoter sequences of both species, identify binding patterns, and assess sequence degeneracy. This approach revealed both previously known and novel binding sites for FnrN and $FixK_2$, enabling the distinction between direct and indirect targets, and the identification of potential cooperative interactions with other regulators. The detected sites were further characterized using genomic and functional annotations. These *in silico* predictions were experimentally validated through *in vitro* and *in vivo* methods, including protein-DNA interaction assays and gene expression analyses. Our model confirmed the ability of $FixK_2$ to recognize two promoters of FnrN: *Rlvc_fixNOQP* (*cbb*$_3$ terminal oxidase) and *Rlvb_1399* (heat shock protein). This cross-species regulatory activity provides strong empirical support for the predictive performance of the model and suggests a conserved function between FnrN and $FixK_2$, despite their direct or indirect mechanism to perceive the signal. Finally, our approach could be extended to other key regulators of microoxic metabolism, thereby broadening its applicability for mapping transcriptional regulatory networks and advancing their biotechnological potential.

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P2.11

Same strain, different outcome: contrasting effects of Pseudomonas isolates on Brassica napus

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Abstract

In microbial ecology and plant–microbe interaction studies, ecological traits are often inferred from taxonomic identity. Genome similarity thresholds, such as average nucleotide identity (ANI), are commonly used to define species (>95% ANI) and even strain-level relationships (>99.99% ANI) [1], implicitly assuming functional equivalence among closely related isolates. However, ANI reflects only nucleotide similarity across aligned genomic regions and does not capture structural variation, such as differences in gene content, synteny, or the loss of large genomic regions. We hypothesize that even isolates exceeding strain-level ANI thresholds may exhibit divergent ecological behaviors in plant-associated contexts.

Pseudomonas sp. CDVBN10A and Pseudomonas sp. CDVBN10B, originally isolated as root endophytic bacteria from *Brassica napus*, have been described as promising PGPBs for this crop [2,3]. Despite sharing >99.99% ANI, the absence of a large genomic region in strain CDVBN10B compared to CDVBN10A results in pronounced phenotypic and functional differences.

Phenotypic characterization revealed that *Pseudomonas sp.* CDVBN10B exhibits reduced cell size, increased swimming motility, and distinct colony morphology compared to strain A. Although both strains showed similar colonization levels *in planta*, the genomic loss improved plant growth promotion. Transcriptomic analyses revealed strikingly different responses between interactions with isolates A and B, with isolate A triggering a much broader activation, as indicated by a higher proportion of upregulated genes in both plant and bacteria.

Overall, our results demonstrate that even isolates with extremely high genomic similarity (categorized within the same strain) can exhibit substantial functional divergence when structural genomic differences, such as the loss of large genomic regions, are present. These findings highlight that high ANI alone is not sufficient to predict functional equivalence in PGPR–plant interactions, and underscore the importance of considering genome content variation when selecting and optimizing microbial inoculants for agricultural applications.

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P2.12

Metabolic modeling of the ecological processes driving wheat rhizosphere microbiome assembly

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Abstract

Despite the proven efficacy of plant-beneficial bacterial inoculants in promoting plant growth and suppressing pathogens, their establishment in the rhizosphere remains inconsistent under field conditions, largely due to competition with resident microorganisms. Heritable, seed-borne bacteria arrive first to this niche and are therefore likely to play crucial roles in assembling the early rhizosphere microbiome through priority effects. Previous studies have suggested that seed-borne bacteria may pre-emptively consume resources early in the development of the plant rhizosphere, thereby limiting the establishment of late arrivals [1]. At the same time, seed-borne bacteria may also release metabolites that facilitate the assembly of other bacteria. However, the metabolic interactions between seed and soil bacteria remain largely unexplored and could greatly impact new strategies for enhancing the establishment of plant-beneficial inoculants. In this study, we sequenced a collection of around 40 bacterial isolates obtained from wheat seeds, the wheat rhizosphere, and soil. We constructed genome-scale metabolic models and predicted the utilization of common carbon sources found in the rhizosphere. These analyses provide a framework to identify potential competition, niche partitioning, and facilitative metabolic interactions among early- and late-arriving bacteria. Building on these predictions, we are experimentally validating carbon utilization profiles and constructing different synthetic communities (SynComs) to study how seed-borne bacteria influence community assembly. By manipulating the arrival order of seed bacteria and performing drop-out experiments, we aim to determine whether seed bacteria act as major drivers of wheat rhizosphere microbiome assembly through priority effects. This framework will also allow us to test how plant-beneficial inoculants establish within communities shaped by inherited bacteria, providing ecological principles to improve inoculant integration in the rhizosphere microbiome.

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P2.13

Seed-borne bacteria drive the assembly of the wheat rhizosphere microbiome

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Abstract

The rhizosphere microbiome is a major determinant of plant health and productivity, yet the ecological processes governing its assembly remain poorly resolved. Soil has traditionally been regarded as the principal source of rhizosphere bacteria, whereas the contribution of heritable seed-borne bacteria has received much less attention. Here, we investigated the coalescence of soil and seed-derived microbiomes in wheat using a complex and reproducible natural rhizosphere community generated through sequential propagation in a microcosm system. By combining 16S rRNA amplicon sequencing with genome-resolved shotgun metagenomics, we found that seed-borne rhizosphere bacteria surpassed native soil microorganisms as the dominant source of the early wheat rhizosphere microbiome. These results indicate that vertically transmitted bacteria are major ecological drivers of community assembly during early rhizosphere formation. Functional analyses further showed that seed-borne bacteria were enriched in host-associated traits linked to the degradation of key disaccharides, supporting a mechanism of niche partitioning in the rhizosphere. In addition, these bacteria displayed metabolic features consistent with facilitative interactions, including cross-feeding that supported partner strains. In vitro co-culture experiments confirmed that helper seed-borne strains promoted the growth of other bacteria on disaccharides as the sole carbon source. Together, these results show that seed-transmitted bacteria shape wheat rhizosphere microbiome assembly through both resource partitioning and metabolic facilitation [1]. This work highlights microbial inheritance as a central determinant of rhizosphere succession and provides a tractable framework for studying natural microbiome assembly.

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POSTERS

SESSION 3

Molecular Biology and Physiology of Plant–Microorganism Interactions (II).

P3.1

Identification of a cyclic diguanylate-activated gene cluster in *Rhizobium etli*

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Abstract

The second messenger cyclic diguanylate (cdG; c-di-GMP) is a bacterial lifestyle switch molecule, best known for its role in biofilm formation and the production of numerous biofilm matrix components, including many extracellular polysaccharides and proteins [1]. We have previously reported that elevated c-di-GMP levels in *Rhizobium etli* greatly affect the composition of the extracellular proteome, promoting the export of many cytoplasmic proteins to the cell exterior, in addition to proteins involved in adhesion and biofilm formation [2]. A study of the changes in the intracellular proteome induced by artificially elevated cdG levels of this bacterium, allow us to identify several transcriptional regulatory proteins affected by cdG. Among these, we focused on RHE_CH01362, a putative transcriptional regulator of unknown function, whose protein levels were enhanced by cdG. RHE_CH01362 is a putative 2-component response regulator (RR), containing a C-terminus LuxR-type HTH DNA binding domain, and a N-terminus RECeiver domain. However, this REC domain lacks the conserved phosphorylatable Asp residue typical of this class of proteins. The gene encoding RHE_CH01362 is located within a 43,78 Kb chromosomal gene cluster of unknown function. Most proteins encoded in this cluster also appeared enhanced by c-di-GMP in our proteomic study. Quantitative RT-PCR showed that transcription of RHE_CH01362 and most of the surrounding genes is significantly increased by elevated c-di-GMP levels, indicating that activation of this gene cluster by cdG operates at transcriptional level. We have named this gene cluster as *dac* (*d*iguanylate *a*ctivated *c*luster) and are currently studying the role of *RHE_CH01362* and the function of this *dac* cluster.

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P3.2

Regulation of N_2O emissions by the soybean endosymbiont *Bradyrhizobium diazoefficiens*

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Abstract

Nitrous oxide (N_2O) is the third most powerful greenhouse gas due to its high radiative capacity. N_2O emissions are mainly produced by abusive utilisation of synthetic nitrogen (N) fertilizers in agriculture. Denitrification and nitrification by soil microorganisms are the main processes involved in nitrogen fertilizers transformation to N_2O. One strategy to mitigate N_2O emissions is to reduce the dependency of N-fertilization by increasing biological N_2 fixation through legume-rhizobia symbiosis. However, some rhizobia are also able to denitrify under free-living and symbiotic conditions. In the denitrification pathway, the nitrous oxide reductase (Nos) reduces N_2O to N_2. A better understanding of Nos regulation is an emerging issue to mitigate N_2O emissions from legume crops.

In *Bradyrhizobium diazoefficiens*, the soybean endosymbiont, Nos is encoded by the *nosRZDFYLX* operon that is induced by the $FixK_2$ transcription factor in response to low oxygen conditions [1]. However, the control of *nosRZDFYLX* genes by the RegSR-NifA regulatory cascade [2] is unknown. Here, we performed an integrated study of *nosRZDFYLX* expression and Nos activity in *regR*, *regS*$_1$, *regS*$_2$, *regS*$_{1/2}$, and *nifA* mutants cultured under denitrifying conditions. Our results showed that RegR activates expression of *nosRZDFYLX* genes and that $RegS_1$ and $RegS_2$ sensors appeared to play a role in this control. The activating role of RegR in N_2O reduction was validated by using a robotized incubation system that monitors real-time changes in O_2 and denitrification gases (NO, N_2O and N_2). Moreover, we also demonstrated by electrophoretic mobility shift assays (EMSA) that RegR interacts with the *nosRZDFYLX* promoter by binding to a putative RegR box. Further studies are currently underway to improve our understanding of the molecular mechanism of the RegR and $FixK_2$ cross-talk to modulate *nosRZDFYLX* expression in the context of N_2O mitigation.

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P3.3

The bacterial RIN4-Interacting Effector (RIE) determines symbiotic compatibility with soybean hosts

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Abstract

Type-III effectors secreted by bacterial pathogens directly target host cellular pathways to suppress plant immunity and promote infection [1]. However, in the context of root nodule symbiosis, the role of rhizobial effectors in regulating plant symbiotic and defense pathways remains unclear.

Sinorhizobium fredii strains are symbiotically compatible with more than 100 legume species, including the agronomically important soybean [2]. Previous studies have shown that this compatibility is also influenced by Type-III effector delivery, which primarily suppresses plant immunity and allows rhizobia to colonize the host [3].

To understand the molecular mechanisms of *S. fredii* Type-III effectors in the plant host, we identified their targets via a yeast two-hybrid screen using a soybean cDNA library. We found that *RIN4-Interacting Effector* (hereafter RIE) interacts with RIN4, a well-characterized regulator of plant immunity [4]. In fact, a recent study shows that RIN4 is also involved in regulating plant symbiotic signaling for successful rhizobia colonization [5]. We therefore hypothesize that RIE induces post-translational modifications in RIN4, thereby modulating the balance between symbiotic signaling and plant immune responses.

Our work confirmed this hypothesis. We have determined that RIE is secreted and translocated into the plant cell through the Type-III Secretion System, and we have validated RIE-RIN4 interaction *in vivo*. Additionally, our we have demonstrated that RIE is involved in the symbiotic compatibility between *S. fredii* and soybean in a cultivar-dependent manner. In a genetically diverse set of soybean lines, a loss-of-function mutant in RIE caused either enhanced or reduced nodulation relative to the wild-type strain. Together, these findings support a model in which the RIN4-RIE functions as a regulatory hub that integrates immune and symbiotic signaling during the rhizobia-legume interaction.

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P3.4

The Phosphorylated Pathway of Ser Biosynthesis (PPSB) is essential for the rhizobium–legume symbiosis in *Lotus japonicus*

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Abstract

Legumes form a mutualistic association with rhizobia, which reduce atmospheric N_2 into ammonium for the plant. The development of nitrogen-fixing root nodules depends on two tightly regulated host processes: *de novo* organogenesis of nodule primordia and rhizobial colonization[1,2].

The amino acid L-serine (Ser) participates in the biosynthesis of several biomolecules, including one-carbon (1C) units essential for methylation reactions, purine and pyrimidine synthesis, which are critical for cell differentiation and proliferation. In plants, Ser is synthesized mainly through the Phosphorylated Pathway of Ser Biosynthesis (PPSB) and the glycolate pathway. We recently described the Ser–Gly–1C network as a metabolic hub regulating sulfur metabolism and root development[3].

In legumes, sulfur metabolism and root architecture are crucial for symbiotic nitrogen fixation[4]. Here, we characterized *PGDH* (3-phosphoglycerate dehydrogenase) genes in *L. japonicus* and identified three orthologs: *LjPGDH1*, *LjPGDH2-1* and *LjPGDH2-2*. Expression analyses showed that *LjPGDH1* and *LjPGDH2-1* are expressed in roots and nodules.

Using mutant lines from the *LORE1* collection[5], we found that single mutants for *LjPGDH1* (*p1p1*) do not display the embryo-lethal phenotype observed in *Arabidopsis*[6], suggesting functional redundancy. Segregation analysis of crosses between *LjPGDH1* and *LjPGDH2-1* mutants revealed lethality of double homozygous individuals, with deviations from Mendelian expectations pointing to a predominant role for *LjPGDH1*. Phenotypic characterization of individuals derived from double heterozygous lines (*P1p1/P2p2*) revealed a gene-dosage effect: plants homozygous for *LjPGDH1* and heterozygous for *LjPGDH2-1* (*p1p1/P2p2*) showed the greatest biomass reduction, particularly under symbiotic conditions, where nodule organogenesis was compromised.

Metabolome analysis of *p1p1/P2p2* mutants revealed altered Ser/Gly contents, potentially affecting 1C supply and cell differentiation during nodule maturation. Transcriptome analysis showed functional enrichment of genes involved in organ senescence, protein catabolism and cysteine-type peptidase activity, pointing to a premature nodule senescence process and demonstrating the essential role of the PPSB in the establishment of the rhizobium–legume symbiosis.

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P3.5

MYB transcription factors involved in isoflavonoid biosynthesis and nodulation in *Lotus japonicus*

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Abstract

Isoflavonoids are key legume metabolites involved in rhizobial interactions and environmental stress responses. However, the transcriptional networks controlling their biosynthesis and their connection with nodulation remain poorly understood [1]. In this context, several MYB transcription factors (TFs) were previously identified in the model legume *Lotus japonicus* as candidate regulators of isoflavonoid biosynthesis [2].

Here, homozygous mutant lines carrying LORE1 insertions were characterized under abiotic stress conditions to investigate their role in regulating isoflavonoid biosynthesis, together with growth and nodulation parameters after inoculation with *Mesorhizobium loti*. Our results indicate that four of the genes analyzed (*MYB36, MYB13, MYB15* and *MYBS*1) are involved in isoflavonoid biosynthesis depending on conditions [3,4]. In addition, two genes (*MYB52* and *MYBS1*) were associated with nodulation-related processes [5].

These findings suggest that MYB TFs may contribute to both specialized metabolism and nodulation, highlighting their potential role in legume symbiotic traits.

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P3.6

Role of asparagine in the *Lotus japonicus–Mesorhizobium lotis*ymbiosis

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Abstract

Previous studies have highlighted the significance of asparagine in various processes and genes related to nitrogen translocation in the model legume *Lotus japonicus* (Credali et al., 2013). Specifically, differential expression of asparagine biosynthesis genes has been observed across different organs in nodulated plants (García-Calderón et al., 2017).

More recently, the *LjASN1*gene was demonstrated to be crucial for the plant's carbon-nitrogen balance through the analysis of *LORE1*insertion mutants, revealing significant physiological differences between nodulated and non-nodulated plants (Rosa-Téllez et al., 2026). New studies carried out with mutant in the *LjASN2* gene, which is predominantly expressed in nodules, indicate that a deficiency in this gene does not alter the examined nodulation parameters; however, metabolomics analysis showed altered asparagine levels, together with other metabolites as detected via metabolomic analysis.

Building upon the hypothesis proposed by Lodwig, Hosle, and Poole (2003) regarding the role of amino acid flux in nitrogen fixation, we analyze the potential relevance of asparagine in the C-N exchange between the microsymbiont and the host plant.

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P3.7

AuxT, a rhizobial auxin transporter packaged in extracellular vesicles, contributes to efficient symbiosis in *Sinorhizobium fredii* HH103

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Abstract

Cell-derived Extracellular Vesicles (EVs) are key mediators of molecular exchange during plant-microbe interactions, potentially contributing to the establishment and maintenance of the rhizobium-legume symbiosis (Ayala-Garcia et al., 2024). Despite their relevance, the function of many EV-associated proteins remains unknown. In a proteomic analysis of EVs isolated form soybean nodules infected with *Sinorhizobium fredii* HH103, we identified a protein annotated as an auxin efflux carrier (AuxT) showing structural similarity to plant PIN transporters (Ung et al., 2022).

To gain insight into its function, we carried out a characterization of AuxT combining *in silico*, genetic and physiological approaches. Structural modelling and bioinformatic analyses revealed that AuxT is a conserved multipass transmembrane protein with high structural similarity to auxin transporters from both plants and bacteria, and suggested a strong affinity for auxin molecules. Functional analyses based on an *auxT* deletion mutant and a complemented strain demonstrated that AuxT is present in EVs derived from both free-living bacteria and bacteroids, as confirmed by western blot and fluorescence microscopy. A significant reduction in auxin content in EVs from the *auxT* mutant compared to the complemented strain was detected, supporting a role for AuxT in auxin packaging into vesicles. Consistently, symbiotic assays in soybean revealed that the *auxT* mutant exhibits impaired nodulation efficiency and reduced plant growth promotion.

Collectively, these results indicate that AuxT is an EV-associated auxin transporter involved in phytohormone packaging, contributing to plant growth promotion and to the efficiency of the symbiotic interaction between *S. fredii* HH103 and soybean.

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P3.8

Functional analysis of type VI secretion system effectors in *Sinorhizobium fredii* USDA 257

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Abstract

The symbiosis between rhizobia and legumes depends on multiple molecular signaling pathways including various protein secretion systems. Among these, the Type VI Secretion System (T6SS) has recently gained attention for its dual role in interbacterial competition and interactions with eukaryotic hosts, although its function in the rhizobium–legume symbiosis remains poorly understood. Regulatory molecules such as acyl-homoserine lactones (AHLs), the autoinducers of quorum sensing (QS) systems, and the second messenger cyclic di-GMP (c-di-GMP) have been described as modulators of the transition between motility and biofilm formation in plant-associated bacteria. Using β-galactosidase activity assays, we previously demonstrated that elevated levels of c-di-GMP and/or AHLs contribute to the transcriptional activation of the T6SS [1], facilitating its characterization. However, conventional T6SS competition assays showed that in *Sinorhizobium fredii* USDA257 this system does not appear to be involved in bacterial competitiveness as reported for other rhizobia [2]. We therefore adapted these assays to maximize T6SS expression and check its activity under our experimental conditions. In parallel, we explored whether USDA257 T6SS effectors may act on plant cells through secretion into the extracellular medium affecting the cell surface. In silico analysis of the USDA257 T6SS gene cluster identified two putative effector genes (tsre1 and tsrx), in addition to the C-terminal effector domain of the T6SS protein VrgG (FhaB). Based on sequence similarity to NCBI database proteins, FhaB and Tsrx could act extracellularly on the plant cell wall: FhaB may exhibit polysaccharide lyase activity, contributing to bacterial attachment, while Tsrx shows features of a glycoside hydrolase that could degrade xyloglucan and facilitate penetration. To assess their roles, both genes were cloned into salicylate-inducible expression plasmids in *E. coli* for functional characterization, aiming to better understand the involvement of T6SS effectors in rhizobium–legume symbiosis.

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P3.9

From free-living cells to bacteroids: metabolic regulation driving efficient nitrogen fixation in legume symbiosis

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Abstract

Legume–rhizobia symbioses require tight integration of sensory systems, bacterial motility, and carbon and nitrogen metabolism to support successful recruitment, root colonisation, and symbiotic efficiency within root nodules. Here, we investigate metabolic control across free-living and symbiotic stages using the model interaction between *Pisum sativum* and *Rhizobium leguminosarum* bv. *viciae*. We show that nitrogen starvation reduces TCA cycle flux and redirects carbon into carbon-storage polymers such as glycogen, polyhydroxybutyrate, and exopolysaccharide, mediated by the phosphotransferase system PTS^{Ntr}. To understand how bacteroid differentiation influences symbiotic efficiency, we compared near-isogenic *R. leguminosarum* strains nodulating pea (*R. leguminosarum* bv. *viciae* A34) and bean (*R. leguminosarum* bv. *phaseoli* 4292). Although bean plants fix more nitrogen overall due to greater biomass, pea nodules exhibit 1.6–3-fold higher nitrogen fixation per unit nodule mass. This increased efficiency arises from denser bacteroid packing, a greater proportion of infected nodule volume, and enhanced allocation of bacteroid protein toward nitrogenase, associated N_2 fixation pathways, and dicarboxylate metabolism. Our results indicate that plant-driven bacteroid differentiation, likely mediated by antimicrobial peptides, optimises metabolic investment in nitrogen fixation.

Together, these findings demonstrate that rhizobial success, from motile free-living soil bacteria to highly efficient symbiotic bacteroids, is shaped by the integrated regulation of sensory systems and central metabolism, highlighting key determinants of effective symbiosis and rhizobial inoculant performance.

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P3.10

Mechanistic insights into *Arabidopsis* growth promotion by the *Sinorhizobium meliloti* volatilome

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Abstract

The alfalfa endosymbiont *Sinorhizobium meliloti* (Sm) releases several volatile compounds with notable biological activities, which could extend the beneficial effects of Sm beyond nitrogen fixation in leguminous plants [1, 2]. The volatile blend or volatilome emitted by Sm shows phytostimulatory effects on the model plant *Arabidopsis thaliana*, inducing significant increases in both shoot and root biomass and promoting the formation of lateral roots. *Arabidopsis* plants exposed to the Sm volatilome also exhibit increased chlorophyll content and enhanced photosynthetic efficiency. In addition, plant iron-deficiency responses such as rhizosphere acidification and activation of the high-affinity iron transporter IRT1 have been detected in the roots of plants exposed to Sm volatile blends. However, the behaviour of two *Arabidopsis* mutant lines defective in IRT1 demonstrate that an enhanced iron-acquisition capacity of the root system is not responsible for the Sm volatilome-mediated phytostimulatory effect. The transcriptome profile of *Arabidopsis* seedlings exposed to rhizobial volatiles has been determined by RNA-Seq. Results from these genome-wide transcriptional analyses, together with the behaviour of iron-related genes and plant mutant lines altered in iron homeostasis, suggest that the molecular mechanisms underlying the plant growth-promoting effects of Sm volatiles differ from those previously described for the phytostimulatory effects of volatile compounds from other microorganisms.

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P3.11

Assessing the effects of *Sinorhizobium meliloti* volatile compounds on the microbiome of a non-leguminous plant

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Abstract

Bacteria emit blends of volatile compounds (VCs) known as volatilomes [1,2]. Bacterial VCs are well known for their beneficial effects on plants, including promoting growth and enhancing resistance to biotic and abiotic stresses. They can also play important roles in microbe-microbe interactions by affecting growth and bacterial behaviors such as virulence, or stress and antibiotic resistance [1]. Despite these important effects, very little is known about the impact of bacterial VCs on plant microbiomes, which are crucial for plant health. In the case of rhizobia, knowledge about the nature and the biological/ecological roles of the VCs they produce remains scarce [2]. Among the VCs emitted by *Sinorhizobium meliloti* (Sm) are volatile methylketones (MKs), one of which acts as an infochemical between bacteria and hinders plant-bacteria interactions [3]. Recently, we investigated the impact of MKs and Sm volatilomes on *Medicago truncatula*-associated bacterial communities and found that Sm volatilomes maintain *Ensifer* abundance in the root endosphere [4]. Here, we present the effects of MKs and Sm volatilomes on the bacterial populations in the rhizosphere, root endosphere, and phyllosphere of the model plant *Arabidopsis thaliana*.

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P3.12

Secretome-guided analysis of T6SS uncovers novel effectors and conserved effector-cluster modules in *Pseudomonas*

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Abstract

The type VI secretion system (T6SS) is a nanomachine, widely distributed in Gram-negative bacteria, that translocates effector proteins into target cells[1]. These effectors are usually found in effector-immunity pairs to prevent self-poisoning[2]. In this study, the secretion of effectors to their corresponding T6SS cluster is identified. *P. ogarae* F113 secretome proteomic shows that under standard *in vitro* conditions, the F1- system is the dominant functional T6SS, while the other systems remain silenced or inactive. Using this strategy, five effectors associated with F1-, Tfe1, Tfe2, Tfe3 and two new orphan effectors, Tfe9 and Tfe10, have been detected. Tfe10 is a Tae4-type amidase effector and Tfe9 is an ortholog of Tse4 from *P. aeruginosa* PAO1. Competition assays against *Escherichia coli* verify the contribution of these effectors to antibacterial activity, and interaction analyses support effector-immunity specificity and functionality. Finally, the work integrates a comparative analysis in the *Pseudomonas* genus, supported by previous phylogenomic classifications of T6SS clusters[3], identifying non-random associations between effector orthologues and cluster subtypes, which could allow for the analysis of possible specificity in secretion between clusters and effectors.

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P3.13

Exploring the molecular roles of Type-III effectors in symbiotic compatibility between rhizobia and soybean

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Abstract

To establish nitrogen-fixing symbiosis with host legumes, rhizobial bacteria must bypass the plant immune system [1]. Similar to plant pathogenic bacteria, many rhizobial strains suppress plant defenses by delivering effector proteins into host cells via the Type III Secretion System [2]. However, these protein effectors do not always suppress plant immunity. Plants have evolved host mechanisms that can directly or indirectly recognize Type-III effectors, blocking bacterial infection through the activation of effector-triggered immunity (ETI) [3]. The molecular mechanisms by which plants recognize rhizobial effectors remain poorly understood.

Sinorhizobium fredii HH103 is a promiscuous strain symbiotically compatible with the agronomically important soybean cultivar Williams 82 (W82). We hypothesize that HH103's symbiotic compatibility and nodulation phenotypes are driven by evolutionary processes involving the recognition of Type-III-secreted effectors. Our goal is to investigate the role of the HH103 effectors in the soybean host.

To identify the role of these effectors during symbiosis, we have identified their targets in W82 through yeast two-hybrid screening. This work has revealed a list of candidate targets, including proteins involved in plant immunity such as ethylene-response transcriptional regulators, as well as symbiotic and nodule development-related genes, which may be relevant for root-nodule symbiosis. Future work will elucidate the precise molecular mechanisms by which these effectors facilitate symbiosis during nodule development. Understanding the molecular functions of these effectors could be relevant for enhancing legume-rhizobia interactions in agriculture.

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POSTERS

SESSION 4

Agronomic and Ecological Uses of Plant–Microorganism Interactions.

P4.1

Advances in asparagine metabolism in soybean.

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Abstract

Studies conducted in our laboratory revealed significant differences in growth and asparagine accumulation in mutants of the model legume *Lotus japonicus* affected in specific asparagine synthetase (ASN) isoforms (1,2). This model legume species has three different *ASN* genes, which show different tissue expression patterns as well as different roles depending on the N nutrition of the plant (1,3). Some of the characteristics of *L. japonicus* were of special interest from an agronomical point of view 1,2). For this reason, we decided to study the ASN gene family in the cultivated legume soybean. Thanks to the high synteny existing among legume species, and to the fact that *L. japonicus* and soybean share the capacity to form determinate nodules for biological dinitrogen fixation, advances with the model specie can be used to underpin new study with soybean, which is more challenging for genetic manipulation due to its genome size and polyploidy. Bioinformatic and tissue-specific expression analyses were used to characterize the ASN family in soybean, and the most probable orthologous to the three *L. japonicus ASN* genes were identified. CRISPR/Cas9 was used to tackle the interruption of these orthologous genes in soybean, and we are currently screening different generation of transformed plants to look for gene edition events. The current state of this research project and the future perspective will be discussed.

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P4.2

Improving the sustainability of mineral fertilizers partially replacing P and K sources with bio-based alternatives and adding microbial plant biostimulants

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Abstract

The fertiliser industry has been challenged to develop efficient strategies for recycling nutrients in order to produce bio-based mineral fertilisers, which involve replacing some of the conventional, non-renewable raw materials with bio-based ones [1]. However, the bioavailability of nutrients to crops from these alternative bio-based sources is reduced during the initial stages of crop growth due to a lower release rate of nutrients in the early stages [2]. It has been demonstrated that including microbial plant biostimulants (MPBs) in mineral fertiliser formulations improves nutrient use efficiency and crop performance [3]. The starting hypothesis for this work is that adding MPB to a mineral fertiliser in which some of the conventional raw materials have been replaced with a bio-based source compensates for the loss of nutrient bioavailability. To this end, a 9-23-12 mineral fertiliser was prepared using conventional raw materials, but with KCl and H_3PO_4 partially replaced by chicken litter ashes as a source of K (20% replacement) and P (5.2% replacement). One field trial was carried out in Portugal with maize. The treatments were a blank control, a conventional 9-23-12 treatment, a bio-based 9-23-12 treatment and a bio-based 9-23-12 treatment amended with a *Pseudomonas koreensis* strain belonging to IQUIMAB (ULE research group). The objective was to evaluate the agronomic behaviour, in terms of crop yield, of the bio-based mineral fertiliser with and without MPBs, as well as its effect on alpha and beta soil diversity compared to conventional fertiliser. Our results indicate that conventional fertiliser can be replaced by bio-based fertilisers supplemented with MPBs, which increased crop yield compared to conventional mineral fertiliser. Soil microbiome diversity (evaluated using a metataxonomic approach) was unaffected or slightly increased by the new fertilisers. Using these fertilisers aligns with the principles of the circular economy and promotes sustainable agricultural practices.

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P4.3

Enhancing Montado Sustainability through Legume-Rhizobia Inoculation Strategies

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Abstract

The Montado ecosystem is a Mediterranean agro-silvo-pastoral system with high socio-economic value, that covers approximately one third of the forest area in Portugal. However, this unique ecosystem is particularly vulnerable to climate change that poses a major risk for its productivity. To mitigate these effects pasture legumes have been introduced as a management practice as they promote overall soil health and fertility [1]. In this sense, it is important to study the diversity and symbiotic performance of the nitrogen-fixing rhizobia of the legume species, since nodulation by ineffective rhizobia can be detrimental for the pasture system. In this study, we aimed to assess the performance of different rhizobia-based bioinoculants under varying fertilization levels in a field trial (pasture) in a Montado region in Southern Portugal. We evaluated three different treatments during time: INIAV inoculant for legumes paired with two different grass-targeting PGPR strains (Treatment A and Treatment B) and a commercial inoculant (Treatment C). The rhizobial population of the different treatments was evaluated by the plant infection method. The results revealed that treatment C had the highest concentration of rhizobia, while the population in treatment B was the most efficient in fixing nitrogen. We subsequently isolated these rhizobia from the root nodules of *Trifolium subterraneum* and sequenced their 16S rRNA gene. This analysis showed that all isolates belong to the *Rhizobium leguminosarum* species and that we could also recover strains from the INIAV inoculant. Six different strains were selected and showed promising activities for plant growth promotion in plant inoculation and *in vitro* phosphate solubilization assays. Soil 16S rDNA metabarcoding revealed a high bacterial diversity for all treatments. Treatment A, during the second year of the field assay, revealed the highest concentration of *Rhizobium* strains. Altogether these findings provide valuable insights for developing sustainable management practices for the Montado ecosystem.

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P4.4

Indigenous rhizobacteria from saline agricultural soils of La Rábita (Granada, SP) as candidates for bioinoculants to improve cucumber (*Cucumis sativus L.*) tolerance to salinity in local crops

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Abstract

Soil salinization is an increasing constraint in coastal agricultural systems due to seawater intrusion into aquifers, leading to elevated salinity in soils and irrigation water. In La Rábita (Granada, Spain), cucumber (*Cucumis sativus*), a salt-sensitive crop with a threshold of approximately 2.5 dS/m, suffers significant reductions in growth and productivity under saline conditions. In this study, we explored the rhizosphere microbiota of cucumber fields affected by salinity to identify locally adapted bacterial strains with potential as bioinoculants. A total of 12 bacterial isolates were obtained and partially identified by 16S rRNA gene sequencing. Under non-saline conditions (LB), the culturable community was dominated by a single unidentified taxon (~55–60%), while Firmicutes accounted for 25–30% of isolates, including *Priestia spp.* (mainly *P. megaterium* and *P. flexa*), *Rossellomorea oryzaecorticis, Terribacillus saccharophilus, Bacillus licheniformis, Cytobacillus firmus* and *Bacillus seohaenensis*. Proteobacteria represented ~15–20%, mainly *Enterobacter ludwigii*.
Under saline conditions (LB + 0.5 M NaCl), community composition shifted markedly, with Firmicutes increasing to ~70–80%, indicating strong selection for halotolerant Bacillota. Several isolates, particularly *P. megaterium, T. saccharophilus* and *B. licheniformis*, were enriched under salinity. Functional screening revealed substantial variability in antioxidant activity, with the highest levels observed in *P. megaterium* (66.1% inhibition), followed by *R. oryzaecorticis* (~35.4%) and *B. seohaenensis* (~31.4%). Under saline conditions, selected strains also showed enhanced production of osmoprotectants, including trehalose (P. megaterium 1.18 µg/mL; *B. seohaenensis* 0.86 µg/mL; *T. saccharophilus* 0.83 µg/mL) and proline (*P. megaterium* 12.44 µg/mL). Selected strains were further evaluated as biotreatment in cucumber seedlings under salt stress, resulting in improved plant development, biomass and photosystem II efficiency. These findings highlight the potential of locally adapted rhizobacteria as sustainable bioinoculants to mitigate salinity stress in coastal cucumber production systems.

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P4.5

New species of *Pseudomonas* from estuarine soils with biofertilizer potential under stress conditions

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Abstract

The estuaries of the Tinto, Odiel, and Piedras rivers in Huelva (SW Spain) have been extensively studied because of their ecological importance. Issues such as heavy metal contamination, nutrient deficiency, and degradation due to climate change require immediate action. To help restore these ecosystems, a large collection of plant growth-promoting bacteria (PGPB), including several identified as new species, was isolated from the rhizosphere and tissues of native plants. In this study, we analyzed the strains N4, N8^T, L1^T, and SDT3^T as potential new species. A phylogenomic analysis showed that all four strains belong to the genus *Pseudomonas*, forming distinct, well-supported clades. Digital DNA-DNA hybridization and average nucleotide identity values were below the species delineation thresholds (70% and 95–96%), confirming that strains N8^T, L1^T, and SDT3^T are novel, while N4 and N8^T are the same species. Consequently, we propose the names *Pseudomonas medicaginis* sp. nov., *Pseudomonas onubensis* sp. nov., and *Pseudomonas spartinae* sp. nov., with *P. medicaginis* consisting of two strains. These strains, identified as PGPB in previous works, contained genes related to PGP traits and were further characterized phenotypically and biochemically. To assess the agroecological potential of the N8^T strain as a nodule enhancer, it was tested in co-inoculation with the corresponding rhizobia on lentil, pea, alfalfa, and bean under greenhouse conditions. In all legumes, N8^T improved shoot and root biomass, increased nodule numbers, and enhanced nitrogen content compared to single rhizobia inoculation. In summary, these estuarine ecosystems are not only ecologically significant due to their microbial diversity but also act as reservoirs of beneficial bacteria with promising agricultural applications [1].

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P4.6

Soil microbial responses to climate-smart management practices in Mediterranean dehesas

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Abstract

Mediterranean dehesa are high-value silvopastoral ecosystems in which microbial communities play a key role in carbon sequestration, nutrient cycling, and the regulation of greenhouse gas (GHG) emissions. Through these processes, soil microbiota strongly influences the capacity of these systems to mitigate and adapt to climate change [1]. However, land-use abandonment and increasing climatic stress threaten their ecological functioning and long-term sustainability. In this study, we investigate how habitat type, climate, and soil management practices shape soil microbial communities, with a view to assessing their contribution to CO_2 sequestration and other greenhouse gas-related processes in forthcoming years. Field experiments were established in two dehesa systems located in contrasting climatic regions. Within each site, experimental plots were established under tree canopy and in adjacent open grassland areas to assess habitat effects. In each habitat, pasture productivity and soil functioning were manipulated through a factorial design combining legume enrichment [2], and four soil management treatments: (i) control, (ii) manure fertilization, (iii) biochar application, and (iv) combined biochar and manure application. These treatments were implemented to evaluate these management practices as a sustainable strategy to enhance carbon sequestration and regulate nutrient availability in dehesa soils [3]. To determine whether these interventions translate into measurable ecological changes, soil microbial communities have been characterized using phospholipid fatty acid (PLFA) profiling, extracellular enzymatic activities and high-throughput amplicon sequencing. By integrating microbial taxonomic composition, functional indicators, and management effects across contrasting climatic contexts, this study advances our understanding of the role of soil microbiology in supporting climate-resilient and sustainable dehesa livestock systems.

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P4.7

Urtica urens endophytes increase plant development without compromising phytochemical yield

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Abstract

Urtica urens (dwarf nettle) is a bioactive annual herb belonging to the *Urticaceae* family and rich in phenolic compounds, including flavonoids (kaempferol, quercetin, isorhamnetin, and rutin glycosides) and phenolic acids (caffeic, chlorogenic, and sinapic acids) [1,2]. While its therapeutic potential is linked to recognized antioxidant, anti-hyperglycemic, cytotoxic, and antimicrobial properties, specific research remains scarce, as it is frequently overshadowed by the well-studied *Urtica dioica*. Similarly occurs with the study of its endophytes, with a significant knowledge gap regarding its associated microbiota, with no previous studies characterizing the *U. urens* microbiome.

In this study, wild *U. urens* specimens were collected in Salamanca (Spain). Foliar endophytic bacteria were isolated across multiple media, yielding 19 isolates belonging to 8 distinct genera. Selected strains exhibited plant growth-promoting (PGP) traits, including siderophore production, phosphate solubilization, and phytopathogen inhibition. Inoculation assays demonstrated differential growth-promoting effects on both aerial and root systems at 28 days post-inoculation (dpi). Furthermore, specific strains enhanced plant resilience against the pathogen *Fusarium* sp.

Phytochemical profiling confirmed the synthesis of key metabolites such as quinic, ferulic, chlorogenic, and *p*-coumaric acids, alongside pinene-ol-O-glucoside, 3,4-(methylenedioxy) cinnamic acid, japonic acid, 9-hydroxy-10,12-octadecadienoic acid, isorhamnetin, and 3-O-rutinoside. Crucially, inoculated plants maintained secondary metabolite profiles comparable to uninoculated controls. These findings suggest that these novel endophytes can be leveraged to boost plant health and defence without compromising the host's natural phytochemical yield.

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P4.8

Lupin based meat substitutes obtained from drought tolerant cultivars with high nitrogen fixing capacity

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Abstract

Livestock is responsible for the increase in greenhouse gas (GHG) emissions, making the production of protein of animal origin unsustainable. In this context, legumes can contribute to the protein needs of humans and reduce GHG emissions by their capacity to fix atmospheric nitrogen in symbiosis with soil bacteria [1]. Among legumes, *Lupinus* species are suitable to produce protein concentrates because of their composition of amino acids and their agronomic adaptations to mediterranean climate, which make of them an alternative to soya, thereby reducing Europe's dependence on this source of protein [1]. Within the genus *Lupinus,* three species (*L. albus, L. angustifolius, L. luteus*) are of great agronomic interest, although they differ significantly in their potential uses and soil and climate requirements. The objective of this study is to select cultivars with the aim to promote lupins as an emerging source of protein. To this end, grain yield, drought tolerance, and nitrogen-fixing capacity have been analysed in a study conducted on soils from an IFAPA experimental farm (Camino de Purchil s/n, Granada).

Seeds of eight cultivars of three *Lupinus* species were nutritionally characterized, revealing significant variability among species and cultivars [2]. Three varieties of *L. albus* showed the best nutritional profiles and the highest grain yield in a field trial. Additionally, the nodular biomass was almost double in *L. albus* compared with *L. luteus* and *angustifolius* showing also high drought resistance. Therefore, *L. albus* cultivars were selected for the identification of oxidative stress markers, transpiration rate and nitrogenase activity under drought conditions.

Finally, the production of protein isolates from lupin seeds resulted in protein isolates with excellent technological properties, enabling the development of low GHG meat substitutes with proteins directly obtained from biological nitrogen fixation process.

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P4.9

Plant Growth Promoting Vesicles: Molecular Inoculants to Enhance Rhizobium-Legume Symbiosis

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Abstract

Extracellular vesicles (EVs) are proteolipidic structures that facilitate interdomain communication by delivering signalling molecules to target cells. Despite the importance of the rhizobium-legume symbiosis, the role of rhizobial EVs as carriers of symbiotic signals remains largely unexplored. This study aimed to identify and quantify Nod Factors (NFs) within EVs from *Sinorhizobium fredii* HH103 and evaluate their potential as molecular inoculants. Characterization of HH103 EVs revealed that induction with the flavonoid genistein triggers hypervesiculation, significantly increasing the production of EVs loaded with high molecular weight NFs. Biological validation through nodule primordia visualization and growth chamber assays in *Glycine max* and *Lotus japonicus* demonstrated that these NF-loaded EVs promote nodule formation. Conversely, EVs from non-induced cultures did not increase nodule numbers but did improve general plant growth parameters. A two-year field experiment in Southern Brazil further confirmed that the application of NF-loaded EVs significantly boosts soybean crop yield, particularly in the absence of water deficit. These findings highlight the role of rhizobial EVs in enhancing symbiotic interactions and suggest their use as a sustainable agricultural strategy to optimize legume productivity.

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P4.10

Towards healthy soils: A phytoremediation strategy for metals using legumes and rhizospheric bacteria

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Abstract

This research project addresses soil degradation resulting from mining activities, specifically focusing on the "Cabeza de los Gatos" waste dump, where high concentrations of metals such as lead, arsenic, copper, and zinc inhibit plant growth and deplete microbial populations [1]. The primary objective is to develop a phytoremediation tool centered on the symbiotic interaction between the Mediterranean legume *Medicago polymorpha* and its rhizospheric microbiota.

The methodology integrates metagenomics and culturomics to analyze and isolate bacterial strains with plant growth-promoting (PGP) properties. A significant aspect of this project is the potential discovery and description of previously uncultured bacterial species through advanced culturomic techniques [2]. Selected isolates will be organized into bacterial consortia to evaluate their efficacy in enhancing plant biomass and remediation capacity under greenhouse conditions using contaminated soils. Furthermore, the most effective consortium will be subjected to additional abiotic stressors—drought and high temperatures—to simulate the harsh summer conditions of the affected region [3].

To evaluate the success of the treatment, the study will monitor photosynthetic parameters, biometric traits, and antioxidant stress responses, alongside the quantitative reduction of metals in the soil. A critical safety parameter involves assessing the translocation of metals to the aerial parts of the plant to ensure that toxic elements do not enter the food chain through livestock grazing [3].

Finally, the project seeks to elucidate the fundamental mechanisms of these interactions. By generating knockout mutants of isolated bacteria to eliminate ACC deaminase activity, researchers will investigate the specific role this enzyme plays in mitigating stress caused by heavy metals, desiccation, and thermal extremes. This mechanistic approach aims to provide deeper insights into how microbial properties facilitate plant resilience in degraded environments.

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P4.11

Optimization of Clonal Rooting in Hemp (*Cannabis sativa* L.): Plant Growth-Promoting Bacteria as an Alternative to Synthetic Auxins

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Abstract

Hemp (*Cannabis sativa* L.) cultivation has expanded rapidly due to its industrial, medicinal, and agronomic relevance, requiring efficient clonal propagation systems to ensure genetic uniformity and crop performance. Vegetative propagation through stem cuttings is widely used; however, rooting success remains highly variable and dependent on genotype, substrate, and environmental conditions [1]. Exogenous auxins, particularly indole-3-butyric acid (IBA), are commonly applied to enhance adventitious root formation, often increasing rooting efficiency by up to an order of magnitude compared to untreated controls [2]. Nevertheless, inconsistent responses among cultivars and regulatory restrictions on hormone use in some production systems highlight the need for alternative approaches [3].

This study aims to optimize rooting systems for hemp cuttings and evaluate plant growth-promoting bacteria (PGPB) as an alternative to synthetic hormones. Propagation protocols have been standardized using jiffy pellets under sterile (autoclaved) and non-sterile conditions, assessing their impact on rooting performance. Additional strategies have been implemented to prevent cross-contamination between alveoli, addressing a key limitation in clonal propagation systems. Finally, selected bacterial strains with the ability to produce auxins (e.g., indole-3-acetic acid, IAA) have been tested as biostimulants for root induction.

Our results show that several bacterial strains produce the same rooting percentages than the positive control (synthetic hormone), and that both, the protocol and the bacterial strain, depend on the hemp genotype.

Such microorganisms represent a promising alternative, as auxin-producing rhizobacteria can stimulate root development while potentially enhancing plant health and sustainability.

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P4.12

Inoculation of *Lavandula officinalis* Chaix with selected arbuscular mycorrhizal fungi and plant growth promoting bacteria isolated from black truffle producing trees

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Abstract

Black truffle is one of the world's most prized edible fungi, forming ectomycorrhizal symbiosis primarily with holm oaks. Agroforestry truffle orchards take several years before becoming productive, making the introduction of a secondary crop a valuable strategy for getting additional income. Intercropping with medicinal and aromatic plants, such as *L. officinalis*, which is widely appreciated for its therapeutic properties and essential oil production, represents a promising and commercially viable option.

In this study, we assessed the effects of inoculation with arbuscular mycorrhizal fungi (AMF) and seven plant growth-promoting bacteria (PGPR), isolated from truffle-producing trees, on the growth and essential oil composition of lavender, in a nursery-factorial experiment. Plant biometric parameters were measured and essential oil composition analysed by gas chromatography-mass spectrometry.

Co-inoculation with AMF and some PGPR strains significantly increased aboveground biomass and inflorescence production in lavender compared to non-inoculated controls, although total essential oil yield remained unchanged. Notably, inoculation induced substantial qualitative shifts in the essential oil metabolic profile. In particular, significant reductions in linalool and linalyl acetate, alongside relative increases in compounds such as L-borneol and eucalyptol were observed in plants inoculated with AMF or certain PGPR strains. No consistent patterns due to co-inoculation with both fungi and bacteria were detected. These changes in essential oil composition suggest a reprogramming of plants secondary metabolism triggered by symbiotic interactions with the microbial inoculants, leading to the production of alternative chemical profiles.

Overall, our findings indicate that inoculation with selected microorganisms can modulate both growth and essential oil properties in *L. officinalis*, possibly linked to plant defence or adaptive responses. This approach may offer opportunities to diversify the industrial applications of lavender. Moreover, it represents a promising biotechnological strategy to enhance the functional quality of lavender cultivation, while providing an additional source of income in agroforestry truffle plantations.

Funding

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P4.13

Biological Legacies and Fungal Resilience: The Role of "Perch Trees" as Nucleation Hubs for Mycorrhizal Recolonization in Burned Ecosystems

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Abstract

Wildfire regimes are intensifying globally, with high-severity fires causing critical impacts on both above- and below-ground ecosystem components. In fire-prone Mediterranean forests, extreme soil temperatures during severe wildfires can lead to "soil sterilization," significantly reducing the abundance of mycorrhizal inoculum necessary for natural forest recovery. This study investigates the role of "perch trees", individual survivors within high-severity burn areas, as essential biological legacies that preserve soil microbial diversity and foster mycorrhizal recolonization.

Using a space-for-time substitution approach across two replicated chronosequences (<3, 5-7, and >10 years post-fire) in Central Spain, we analyzed the spatiotemporal dynamics of fungal communities. Specifically, we characterized the taxonomic and functional composition of root-associated mycorrhizal communities within surviving perch trees and compared them to the surrounding soil communities along 100m transects.

We hypothesize that these surviving trees act as spatial anchors and primary reservoirs of fungal diversity, maintaining active mycorrhizal networks that serve as a source of inoculum for the surrounding sterilized soils. We expect to find a strong spatial gradient, where mycorrhizal richness and community similarity between roots and soil decrease with distance from the perch tree, reflecting a nucleation pattern of recolonization. If these hypotheses are supported, it would demonstrate that surviving trees are essential for maintaining soil microbial resilience, highlighting the urgent need for post-fire management policies that prioritize the retention of biological legacies to support nature-based forest restoration and the recovery of plant-microbe interactions.

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P4.14

Global restructuring of soil microbiomes by land use and agricultural inputs

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Abstract

Soil microbiomes play a fundamental role in ecosystem functioning, yet the combined effects of climate and agricultural management on their diversity and organization are still not fully understood at a global scale. In this study [1], we examined 1,921 soil samples collected across 33 countries and spanning a wide range of biomes to evaluate how climatic gradients and agricultural inputs—such as fertilizers and pesticides—shape prokaryotic and fungal communities.

Our analyses revealed that microbial diversity is highest at intermediate temperature ranges and shows clear differences between natural and agricultural systems. Agricultural practices were associated with increased overall microbial diversity, but also with substantial shifts in community composition and ecological guild structure. Specifically, pesticide application was linked to reductions in bacterial diversity and changes in fungal functional groups, whereas fertilization altered microbial network architecture, with organic and inorganic inputs driving distinct responses.

Together, these results highlight that climate and agricultural practices act in concert to structure soil microbiomes, influencing not only diversity but also community organization and network connectivity. These patterns have important implications for soil health and ecosystem resilience in managed environments. Overall, our findings suggest that agricultural interventions—particularly pesticide use and different fertilization strategies—function as strong ecological filters, reshaping soil microbiomes worldwide by increasing apparent diversity while promoting more fragmented, less mutualistic, and potentially less resilient communities.

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P4.15

PGPB from *Typha domingensis* spontaneously growing in extremely Fe-rich substrates

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Abstract

Acid mine drainage (AMD) is water that, after interacting with certain metallic sulfides (primarily pyrite), drops in pH to acidic levels and shows a significant increase in dissolved metallic species. This water causes contamination and deterioration of groundwater and surface streams in the Odiel River Basin. In search of ecological solutions, the LIFE-ETAD project (Andalusian Agency for Environment and Water, University of Huelva, and Sacyr) was developed a few years ago. A passive AMD treatment system was constructed on a publicly owned site known as Mina Concepción. This system includes a series of ponds and reactive tanks designed to raise water pH and precipitate metallic species. Remarkably, aquatic macrophytes (*Typha domingensis*), highly tolerant to high metal/loid concentrations, spontaneously grew in extremely Fe-rich substrates (38–44% of Fe_2O_3) from different components of this acid mine drainage disperse alkaline substrate passive treatment [1]. This spontaneous growth could serve as an environmental indicator of the system's effectiveness but could also be used as a supplementary decontamination step to help accelerate the cleanup process. In this work, rhizospheric and endophytic bacteria from *Typha domingensis* plants growing in several ponds and reactive tanks have been isolated and characterized to develop bacterial consortia that enhance plant growth and metal uptake capacity.

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POSTERS

SESSION 5

Effects of Biotic and Abiotic Stresses on Beneficial Plant–Microorganism Interactions.

P5.1

Enhanced Soil Biomass and Diversity of Arbuscular Mycorrhizal Fungi under Prolonged Warming and Drought Conditions

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Abstract

Arbuscular mycorrhizal fungi (AMF) support critical ecosystem services including plant resource acquisition and productivity [1]. AMF functional traits such as relative biomass investment in root versus soil colonization or drought tolerance are thought to be evolutionarily conserved within AMF lineages, and might influence AMF community responses to climate change[2].
In a long-term field experiment, six coexisting native shrub species were exposed to 9 years of simulated climate warming (2ºC), rainfall reduction (30%) or their combination in a semiarid shrubland.
Photosynthesis and aboveground plant biomass growth were reduced by warming combined with rainfall reduction, while both AMF biomass and diversity in soil increased markedly. In particular, the richness of virtual taxa of the Glomeraceae lineage increased steeply with topsoil desiccation, resulting in overwhelming dominance over other AMF lineages under warming combined with rainfall reduction.
Higher AMF biomass and diversity in soil under warming combined with rainfall reduction suggests increased carbon investment in mycorrhizal fungi by climatically stressed host plants. High tolerance to soil drying appears to be widespread across the Glomeraceae, which may enable AMF in this lineage to buffer the impacts of a drier climate on host plants through effective phosphorus acquisition from dry soil and enhanced plant water use efficiency.

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P5.2

Enhancing white lupin tolerance to chromium through inoculation with native PGP rhizobacteria

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Abstract

Chromium is released into the environment through a range of industrial activities, resulting in the contamination of agricultural soils. Among sustainable remediation approaches, phytoremediation of heavy metal-contaminated soils with white lupin (*Lupinus albus*) has shown great potential, as increased mercury tolerance and hyperaccumulation have been reported in white lupin plants inoculated with a mercury-tolerant *Bradyrhizobium* strain [1].
White lupin trap plants were grown in pots containing soil obtained from a chromium mine. Sixty-two strains were isolated from nodules of these plants and 214 from rhizosphere of root and cluster root segments. Plant growth-promoting (PGP) activities of these strains were tested *in vitro*, specifically N fixation, P solubilization, siderophore and IAA production, and ACC deaminase activity. Twenty-two nodule-associated strains were selected and used to inoculate white lupin (cv. Orden Dorado) seeds, both in presence and in the absence of chromium in the nutrient solution. With the aim of assessing the PGP effect of the strains in the absence and presence of Cr, various plant growth parameters were measured five weeks after sowing: aerial part fresh and dry weight, fresh and dry root weight, leaf area and chlorophyll. Number of nodules per plant, nodule fresh weight and nitrogenase activity were also registered when nodulation occurred. A preliminary analysis of the results shows that several of the inoculated strains, most of which belong to the genus *Bradyrhizobium,* were able to form nodules in the presence of Cr. One of these strains appears to promote plant growth in such a way that the development of the plants grown in the presence of chromium resembles that of plants growing in the absence of this heavy metal.

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Lupinus albus seeds were kindly provided by the Grain Legumes Team, Extremadura Scientific and Technological Research Center (CICYTEX), Spain.

P5.3

Recruitment of rhizospheric bacteria by white lupin roots in chromium-contaminated soils

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Abstract

Chromium is widely used in various industrial processes, leading to the contamination of agricultural soils. Among sustainable remediation strategies, phytoremediation using plants and their associated microorganisms offers a promising solution. White lupin (*Lupinus albus*), a legume capable of forming symbiotic associations with nitrogen-fixing rhizobia, has shown potential for phytoremediation due to its stress tolerance and its ability to develop specialized cluster roots that enhance both nutrient uptake and heavy metal immobilization. Six different ecotypes of white lupin with varying tolerance to chromium were grown in pots containing soils with two different chromium concentrations. Six weeks after sowing, samples of common and cluster roots rhizospheric soil and bulk soil were collected. Metabarcoding analyses were carried out to investigate if the alpha and beta diversity of the bacterial communities associated to common roots and cluster roots differed, and whether they were influenced by the lupin ecotype or the chromium content of the soils. The Shannon index was significantly higher in the rhizospheric soil samples compared to the bulk soil samples, and in high-chromium soil samples compared to low-chromium soil samples. Regarding beta diversity, soil chromium content and soil type (bulk vs. rhizospheric) appeared to be related to differences in the composition of the bacterial communities. In bulk soil samples, there was a high abundance of ASVs corresponding to the families Moraxellaceae and Burkholderiaceae. These families were much less abundant in the rhizospheric soil samples, particularly in the high-chromium soil. In the rhizospheric samples, the family Comamonadaceae was significantly more abundant in the high-chromium soil, whilst families Burkholderiaceae and Xanthobacteraceae were significantly more abundant in the low-chromium rhizospheric soil.

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P5.4

Let the plant choose: drought-driven recruitment uncovers next-generation inoculants

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Abstract

Microbial inoculant discovery is typically driven by trait-based screening performed outside the ecological context where plant-microbe interactions naturally occur, often resulting in poor field performance. Here, we asked a simple question: what if the plant and its environment did the screening first? We leveraged drought-driven rhizosphere recruitment as a nature-guided strategy to identify candidate beneficial bacteria during early tomato seedling establishment in natural soil microcosms. Comparative cultivation across bulk soil and rhizosphere fractions under well-watered and drought conditions revealed that drought acts as a strong ecological filter. Total recoverable bacterial populations decreased from 90 to 73 CFU g^{-1} dry soil under drought, while community composition shifted markedly. Bacillota representatives such as *Priestia megaterium* and *Peribacillus simplex* increased in relative abundance (up to 16.4%), whereas *Pseudomonas fluorescens* declined to 11%. Strikingly, a previously undetected taxon, *Paracoccus* sp. Q, accounted for 20.5% of isolates exclusively in drought-stressed rhizosphere samples.

Recruitment-prioritized isolates displayed complementary functional profiles. *P. megaterium* showed strong auxin-related compound production and biofilm formation, while *Paracoccus* sp. Q exhibited elevated proline production and maintained ~47% of its root colonization capacity under osmotic stress. In early inoculation assays, drought reduced shoot biomass in non-inoculated plants from 45.4 mg to 17.6 mg, whereas inoculation with *Paracoccus* sp. Q restored growth to 50.4 mg shoot biomass and 9.1 mg root biomass under drought conditions. Genome-informed analyses further supported the ecological relevance of this isolate, revealing gene repertoires associated with osmoprotection, chemotaxis, and oxidative stress response, while genome-scale taxonomy indicated that strain Q represents a distinct lineage within the genus *Paracoccus*. Together, these results demonstrate that drought-driven rhizosphere recruitment can be harnessed as an ecologically grounded framework for inoculant discovery, effectively allowing the plant-soil system to pre-select microbial partners compatible with stress conditions.

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P5.5

Histological evidences of mycorrhizal colonization in *Eucalyptus* roots after nursery inoculation

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Abstract

In order to simulate the difficult conditions to which eucalyptus plants are subjected when they are planted, an extreme survival test was carried out under nursery conditions. Clonal *Eucalyptus globulus* plants were transferred into 2,8 L pots filed with a coarse-textured soil and inoculated with different commercial products: endomycorrhiza tablets (with *Rhizophagus intraradices syn*. *Glomus intraradices* and *Funneliformis mosseae syn*. *Glomus mosseae*), ectomycorrhiza tablets (with *Rhizopogon* sp., *Pisolithus tinctorius*, *Scleroderma verrucosum* and *Suillus* spp.) and *Pisolithus tinctorius* spore's suspension. A controlled-release fertilizer was applied to the control. Plants were maintained 7 days in nursery with regular watering and then transferred to exterior conditions during summer (April to July) and maintained without watering or shading. Plants were periodically assessed for survival, growth, axillary buds and leaves development. Compared to control, treatments with mycorrhiza improved the survival rate. After 78 days, when mean survival rate was about 5%, the plants were immersed in water and the roots gently separated from soil. Root samples were cleared and stained using a modified technique [1] and observed under microscope. The development of typical AM (vesicular-arbuscular mycorrhizae) structures such as aseptate hyphae, arbuscules and vesicle were confirmed. However, no ECM (ectomycorrhizae) development was observed, indicating the need for longer time under nursery conditions for complete ECM development.

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P5.6

Expression of leghemoglobins and phytoglobins in response to nutritional stress and hypoxia in *Lotus japonicus*

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Abstract

The genome of *Lotus japonicus* encodes nine hemoglobins: three leghemoglobins (Lbs) and six phytoglobins (Glbs). These, in turn, include two class 1 Glbs (Glb1-1, Glb1-2), two class 3 Glbs (Glb3-1, Glb3-2), and two unusual hemoglobins tentatively named Glb2-1 and Glb2-2, which display intermediate features of the two other classes [1]. Lbs transport O_2 inside the nodules, whereas Glb1-1 modulates nitric oxide (NO) concentration during symbiosis and in the hypoxic response. The function of other hemoglobins is unknown. To investigate the role of hemoglobins under abiotic stresses, we analyzed the transcriptional response of *Lb* and *Glb* genes in *L. japonicus* plants subjected to hypoxia (waterlogging) and macronutrient deficiency (-N, -K, -Ca, -S, -P). We found that Glb1-1 is the only hemoglobin that is induced by both low oxygen and nitrate in roots and nodules, supporting its role in NO homeostasis. We did not observe any effect of hypoxia on the expression of other Glbs. Phenotypic analysis of *glb1-1* knockout mutants revealed a reduction in root length under hypoxic conditions. The nutrient stress was applied to nodulated and non-nodulated plants grown in plates by adjusting the nutrient content and pH. The most important changes occurred in non-nodulated plants, where *Glb1-1* was strongly up-regulated under K-deficiency and down-regulated under N-deficiency in leaves and roots. Interestingly, these transcriptomic alterations were absent in nodulated plants, suggesting a greater tolerance to nutrient deficiencies. Based on these findings, further research is being conducted using *glb1-1* knockout and Glb1-1 overexpressing lines under hypoxia and -N/-K stress. Our data indicate that Glb1-1 plays a key role in environmental stress. Current experiments are set up to assess the impact of both abiotic stresses on the transcriptomes and proteomes of leaves, roots, and nodules to clarify the underlying regulatory mechanisms.

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III Congreso Beneficial Plant-Microbe Interactions

26-27-28/MAYO/2026

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