XVIII SEFIN OEIRAS '22

BeMiPlant

Beneficial Plant-Microbe Interactions

I Spanish-Portuguese

Congress on Beneficial Plant-Microbe Interactions (BeMiPlant)

October 17-19 2022

ORGANIZATION:

National Institute for Agrarian and Veterinary Research

INSTITUTIONAL PARTNER:

SCAP

SOCIEDADE DE CIÊNCIAS AGRÁRIAS DE PORTUGAL

ORGANIZERS:

Sociedad Española de Fijación del Nitrógeno (SEFIN)

Instituto Nacional de Investigação Agrária e Veterinária (INIAV)

INSTITUTIONAL PARTNER:

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Beneficial Plant-Microbe Interactions
(BeMiPlant)

XVIII National Meeting of the
Spanish Society of Nitrogen Fixation (XVIII SEFIN)

BOOK OF ABSTRACTS

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BeMiPlant
Beneficial Plant-Microbe Interactions

October 17th to 19th, 2022
Oeiras, Portugal

PREFACE / WELCOME

Dear colleagues,

On behalf of the Organizing Committee, you are very welcomed to the **First Spanish-Portuguese Congress on Beneficial Plant-Microbe Interactions (BeMiPlant)** and **XVIII National Meeting of the Spanish Society of Nitrogen Fixation (XVIII SEFIN).**

The Congress will be held at INIAV - Instituto Nacional de Investigação Agrária e Veterinária, I.P., in Oeiras, Portugal, from October 17th to 19th, 2022. The event is organized by SEFIN and INIAV.

This meeting officially initiates a new and hopefully lasting series, the **BeMiPlant** series on Beneficial Microbes for Plants, which greatly expands the traditional SEFIN main scientific scope, nitrogen fixation, to integrate all microbes and modes of action that are helpful for soil, agriculture and forestry, and thereby to environment sustainability.

Besides the Opening and Closing Keynote Conferences and the *Antonio Palomares Award* Conference, the congress program includes six Plenary Sessions and three Poster Sessions, giving broad coverage to the latest scientific advances in this area: 1-Diversity and Ecology of Plant-Beneficial Microbes; 2-Microbial Inoculants for Agriculture and Forestry; 3-Beneficial Microbes for Soil and Environment; 4-Nitrogen-Fixing Systems; 5-PGPR, Mycorrhizae and Microbial Endophytes; and 6-Genetics and "Omics" of Plants and Associated Microbes.

The Congress organization invites you to enrol in this event, where the latest achievements on research, innovation and biotechnological applications of beneficial plant-microbe interactions will be discussed under an holistic approach.

We hope that all participants will enjoy during their stay in Oeiras and will found an excellent atmosphere to discuss about scientific matters, find new collaborations and envisage future directions in the field of the beneficial microorganisms and their associations with plants.

We also expect that this meeting will provide new incentives for the successful continuation of the activities of the SEFIN and the Portuguese-Spanish scientific relationships.

Finally, we would like to thank our sponsors: SEFIN, INIAV, SCAP, Oeiras Valley / Município de Oeiras, Fertiprado, ADP Fertilizantes, BioPortugal and Alfagene their invaluable support to this congress.

We wish everyone a most successful and fruitful meeting, and very enjoyable days in Oeiras.

Isabel Videira e Castro
President of the Organizing Committee

Juan Sanjuán
Vice-president of the Organizing Committee

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SCIENTIFIC PROGRAM

Monday, October 17

8.15-9.30 **REGISTRATION** - *Main Auditorium Atrium - Principal Building*

9.30-10.00 **OPENING SESSION** - *Main Auditorium – Principal Building*

Chair: Isabel Videira e Castro

Opening Ceremony

10.00-11.00 **OPENING CONFERENCE**

Antonio Lagares
(Universidad Nacional de La Plata, Argentina)

"Learning on how to live in two contrasting worlds: The case of plant-associated soil bacteria".

11.00-11.30 **COFFEE BREAK** - *CAP Building (1st Floor)*

PLENARY SESSION 1: Diversity and Ecology of Plant Beneficial Microbes

Chairs: Paulo Cardoso and Lorena Carro

11.30-12.00 **CONFERENCE S1-L-01**

Ignacio Vilchez
(ITQB-Nova. Oeiras, Portugal).

"Call-for-help, a potential microbe-shaping biotech in agriculture: from beneficial diversity to soil health recovering ".

12.00-13.00 **ORAL COMMUNICATIONS**

- **S1-O-01:** Espinosa-Saiz D, Paniagua-Gallego F, Velázquez E, García-Fraile P, Mateos PF, Saati-Santamaría Z, **Menéndez E**.
  "Wheat-canola root-associated bacteriomes reveal common and unique taxa with diverse behaviours when combined into synthetic bacterial communities "
- **S1-O-02:** **Castellano-Hinojosa A**, González-López J, Strauss SL
  "Cover crops promote beneficial soil microbiomes but have limited impacts on soil nutrient cycling in citrus orchards"
- **S1-O-03:** **Custódio V**, Salas-González I, Flis P, Amorós R, R. Broadley M, Oliveira MM, Castrillo G
  "The role of soil microbiota and environmental cues in the regulation of maize development"
- **S1-O-04:** **Rocha R**, Lopes T, Fidalgo C, Alves A, Cardoso P, Figueira E
  "Tailoring the cultivable bacterial microbiota as a source of stage-specific biofertilizers"
- **S1-O-05:** **Montero-Calasanz MdC**, Yaramis A, Meier-Kolthoff, JP, Göker M
  "Biotechnological Potential of *Blastococcus* (Actinobacteria) in arid agro-ecosystems"
- **S1-O-06:** **Pulido-Suárez L**, Díaz-Peña J, Notario del Pino, J, González-Rodríguez A, León-Barrios M
  "Diversity of root nodule bacteria associated with *Spartocytisus supranubius* across Teide National Park soils".

13.00-15.00 **LUNCH** - *CAP Building (2nd Floor)*

PLENARY SESSION 2: Microbial Inoculants for Agriculture and Forestry

Chairs: Cristina Cruz and Dulce N. Rodríguez-Navarro

15.00-15.30 **CONFERENCE S2-L-01**

Mónica Montoya
(Universidad Autónoma de Madrid, Spain)

"Bacterial synthetic communities (SynComs) as inoculants for agriculture and environmental protection"

15.30-16:00 **CONFERENCE S2-L-02**

Ana Rincón
(ICA-CSIC. Madrid, Spain)

"Beneficial microbes for forestry applications"

16:00-16.40 **ORAL COMMUNICATIONS**

- **S2-O-01: Carrasco López JA**, Flores Duarte NJ, Navarro de la Torre S, Rodríguez-Llorente ID, Pajuelo, E.
  "Circular agronomy" and Culturomics: Two sides of the same coin in the design of tailored low cost biofertilizers to promote plant growth under abiotic stresses".

- **S2-O-02: Montero-Palmero MB**, Lucas JA, Montalban B, Ramos-Solano B, García-Villaraco A, Gutierrez-Mañero FJ
  "Reverting Chlorosis in iron-starved tomato by PGPB involves a combination of bacterial siderophores and a systemic induction of Fe-uptake responsive genes".

- **S2-O-03: Mazuecos-Aguilera I**, Hidalgo-Castellanos J, Salazar S, Crespo-Barreiro A, Barquero M, Ortíz-Liébana N, López P, González-Andrés F.
  "The combination of rice straw mulch with nitrogen fixers and plant growth-promoting bacteria (PGPB) has a synergic effect in crop production and soil microbiome".

- **S2-O-04: Carpintero JM**, Barquero M, Pinto JC, , Kamah S, González-Andrés F, Branas J
  "A novel complex mineral fertilizer composition including rhizobia improves yield with a reduced dose".

16.40-17.00 **COFFEE BREAK** - *CAP Building (1st Floor)*

17.00-18.30 **POSTER SESSION 1, 2** - *CAP Building (1st Floor)*

19.00-21.00 **WELCOME COCKTAIL**

SCIENTIFIC PROGRAM

Tuesday, October 18

PLENARY SESSION 4: Nitrogen-Fixing Systems

Chairs: Ana Ribeiro and Mª Jesús Delgado

9.00-9.30 **CONFERENCE S4-L-01**

José M. Palacios
(Universidad Politécnica de Madrid, Spain)

"A life in love with science. *In memoriam* Tomás Ruiz Argüeso"

9.30-10.00 **CONFERENCE S4-L-02**

Luis M. Rubio
(CBGP-UPM. Madrid, Spain)

"Engineering nitrogen fixation in cereals"

10.00-10.30 **ORAL COMMUNICATIONS**

- **S4-O-01: Navarro-Gómez C**, Escudero V, Imperial J, González-Guerrero M
  "MtAtx1 is a Cu+-chaperone involved in Symbiotic Nitrogen Fixation"
- **S4-O-02: Minguillón S**, Román A, Becana M, Rubio MC
  "Differential expression of hemoglobins in nodules of the model legume *Lotus japonicus* suggests specific functions"
- **S4-O-03**: Ortíz J, Sanhueza C, Romero-Munar A, Sierra S, Palma F, López-Gómez M, Coba de la Peña T, Aroca R, Bascuñán-Godoy L, **Fernández Del-Saz N**
  "Effect of nitrogen supply and Rhizobia symbiosis in the isotopic composition of essential plant elements, nutrient content, TCA cycle activity and respiratory energy balance of *Lotus japonicus*"

10.30-11.00 **COFFEE BREAK** - *CAP Building (1st Floor)*

PLENARY SESSION 5: PGPR, Mycorrhizae and Microbial Endophytes

Chairs: Isabel Brito and Marta Martín

11.00-11.30 **CONFERENCE S5-L-01**

Etelvina Figueira
(University of Aveiro, Portugal)

"PGPR and bacterial endophytes: Phosphate solubilizing bacteria - where to find?"

11.30-12.00 **CONFERENCE S5-L-02**

Concepción Azcón
(EEZ-CSIC, Granada, Spain)

PLENARY SESSION 5: PGPR, Mycorrhizae and Microbial Endophytes (cont.)

Chairs: Isabel Brito and Marta Martín

12.00-12.30 **ORAL COMMUNICATIONS**

- **S5-O-01**: **Jacott C**, Charpentier M, Murray J, Ridout C
  "Host susceptibility factor MLO facilitates symbiosis with beneficial microbes"

- **S5-O-02: Lopes T**, Cardoso P, Matos D, Rocha R, Pires A, Marques P, Figueira E
  "Graphene oxide influence in soil bacteria is dose dependent and changes at osmotic stress: growth variation, oxidative damage, antioxidant response and plant growth promotion traits of a *Rhizobium* strain"

- **S5-O-03: Flores-Duarte NJ**, Caballero-Delgado S, Pajuelo E, Mateos-Naranjo E, Redondo-Gómez S, Navarro-Torre S, Rodríguez-Llorente ID
  "Enhanced legume growth and adaptation to degraded estuarine soils using *Pseudomonas* nodule endophytes"

12.30-13.30 **POSTER SESSION 4, 5** - *CAP Building (2nd Floor)*

13.30-15.00 **LUNCH** - *CAP Building (2nd Floor)*

15.00-16.00 **CONFERENCE *Antonio Palomares Award***

Chair: Manuel Becana

Marta Robledo (IBBC, CSIC-Universidad de Cantabria, Santander and Biomar Microbial Technologies, Armunia, León, Spain)
"The amazing versatility of molecular tools used by rhizobia to switch between complex lifestyles"

SCIENTIFIC PROGRAM

Wednesday, October 19

PLENARY SESSION 3: Beneficial Microbes for Soil and Environment

Chairs: Helena Machado and Nuria Ferrol

9.00-9.30 **CONFERENCE S3-L-01** — **Jessica Purswani** (Universidad de Granada, Spain)

"Social PGPMs for future biofertilizers"

9.30-10.00 **ORAL COMMUNICATIONS**

- **S3-O-01:** Civantos C, Murillo-Torres M, Velasco-Amo M P, Adrián R, Filloux A, Mavridou D, Landa B, **Bernal P**
  "Type VI Secretion System: a bacterial killing machine and biocontrol weapon"
- **S3-O-02:** Vicente CSL, Espinosa-Saiz D, Curto M, Faria JMS, Velázquez E, Inácio L, Mateos PF, **Menéndez E**
  "Exploring soil Plant Growth Promoting Rhizobacteria potential to control Plant-Parasitic Nematodes: the case of *Phyllobacterium* and *Paenibacillus* against the pinewood nematode *Bursaphelenchus xylophilus"*
- **S3-O-03**: **Reyes Pérez P**, Jiménez-Guerrero I, Sánchez-Reina A, Moreno-de Castro N, Civantos C, Ollero FJ, López-Baena FJ, Bernal P, Pérez-Montaño F
  "Antibiosis or symbiosis? Characterizing the *Sinorhizobium fredii* USDA257 type VI secretion system"

PLENARY SESSION 6: Genetics and "Omics" of Plants and Associated Microbes

Chairs: Margarida Oliveira and Socorro Mesa

10.00-10.30 **CONFERENCE S6-L-01** — **Estíbaliz Larrainzar** (Universidad Pública de Navarra, Pamplona, Spain)

"Is ethylene required for an active nitrogen fixation and nodule development in Medicago truncatula?"

10.30-11.00 **COFFEE BREAK** - *CAP Building (1st Floor)*

11.00-11.30 **CONFERENCE S6-L-02** — **Jose I. Jiménez Zurdo** (EEZ-CSIC, Granada, Spain)

"Structure and function of the α-rhizobia non-coding transcriptome investigated by RNAseq"

11.30-12.30 **ORAL COMMUNICATIONS**

- **S6-O-01**: **del Cerro P**, Cook NM, Huisman R, Dangeville P, Grubb LE, Marchal C, Ho Ching Lam A, Charpentier M
  "Engineered Calmodulin modulates nuclear calcium oscillation and enhances legume root nodule simbiosis"
- **S6-O-02**: Torres DP, Alexandre A, Menéndez E, **Brígido C**
  "Genomic characteristics and comparative genomics analyses of non-rhizobial endophytic bacteria isolated from legumes"
- **S6-O-03**: Fernandes I, Paulo OS, Sarjkar I, Sem A, Marques I, Graça I, Pawlowski K, Ramalho JC, **Ribeiro-Barros AI**
  "Salt stress tolerance in *Casuarina glauca*: insights from the branchlets transcriptome"
- **S6-O-04**: Ayala García, P, Moreno de Castro N, Jiménez Guerrero I, Pérez Montaño F, **Borrero de Acuña JM**
  "Engineering membrane vesicles for fine-tuned modulation of rhizobia species interactions for enhanced nodulation and plant growth"
- **S6-O-05**: De Sousa BFS, Tighilt L, Arrabal A, Domingo-Serrano L, Gómez-Pellicer R, Albareda M, Boulila F, Palacios JM, **Rey L**
  "Relevance of rhizobial T6SS for the interaction with some legumes"
- **S6-O-06**: **Casas-Román A,** Lorite MJ, Muñoz S, Gallegos MT, Sanjuán J.
  "Characterization of the Glyceraldehyde-3-phosphate dehydrogenase protein in *Rhizobium etli"*

PLENARY SESSION 6: Genetics and "Omics" of Plants and Associated Microbes (cont.)

Chairs: Margarida Oliveira and Socorro Mesa

12.30-13.30 **POSTER SESSION 3, 6** - *CAP Building (2nd Floor)*

13.30-15.00 **LUNCH** - *CAP Building (2nd Floor)*

CLOSING SESSION

Chairs: Juan Sanjuán and Isabel Videira e Castro

15.00-16.00 **CLOSING CONFERENCE**

Natalia Requena
(Karlsruhe Institute of Technology, Germany)

"Clues for living together in harmony: the arbuscular mycorrhizal symbiosis"

16.00-16.30 **CLOSING CEREMONY. AWARDS**

16.30-17.00 **COFFEE BREAK** - *CAP Building (1st Floor)*

17.00-19.00 **SEFIN ASSEMBLY** - CAP Building Auditorium (Ground Floor)

20.30-24.00 **CLOSING DINNER**

Bérrio - Restaurante & Terrace
(Avenida Marginal - Praia da Parede, 2770-239 Parede)

SESSION 1: Diversity and Ecology of Plant Beneficial Microbes

S1-P-01 "LegumBiome-Drought Project: Screening of potential plant drought-tolerance enhancers among wild, desert-adapted legumes"
Dias E, McQuade M, Niza M, Jesus HMM, Carrilho J, **Vílchez JI**

S1-P-02 "Evaluation of the microbiota associated with a salt-tolerant rice variety as a treatment to induce resistance in sensitive varieties"
Romão IR, Santos AP, Vílchez JI

S1-P-03 "The rhizosphere microbiome associated with the legume *Spartocytisus supranubius* in the high mountain ecosystem of Teide N.P."
Lizano-Bastardín AL, Villadas PJ, **Pulido-Suárez L**, Fernández-López M, León-Barrios M

S1-P-04 "*Trichoderma* species and arbuscular mycorrhizal populations associated to the banana rhizosphere affected by *Fusarium oxysporum* f. sp. *cubense* in the Canary Islands"
Correa-Delgado R, Pérez-Parrado P, Hernández-Hernández A, Brito-López P, Rodríguez-Cabrera N, Laich F, **Jaizme-Vega MC**

S1-P-05 "Biodiversity of rhizobia-nodulating lentils in Spanish soils"
Martínez Román G, Rodríguez-Navarro DN, de-los-Mozos-Pascual M, Alcántara Ramírez MC, Perea F, Brun P, **Camacho M**

S1-P-06 "Isolation and identification of rhizobia from cowpea nodules *Vigna unguiculata* (L.) Walp. grown on the Peruvian coast"
Valdez-Nuñez RA, Guardia-Medina JR, López-Vega MA, Malvas Herrera KE, Silvera Pablo C

S1-P-07 "Microbiome analysis of wild blackberry and blueberry plants in the northern Iberian Peninsula"
Saati-Santamaría Z, Vicentefranqueira, R, Kolarik M, Rivas R, **García-Fraile P**

S1-P-08 "Following the rhizobia-legume symbiosis in the Montado ecosystem"
Soares R, Munõz O, Fareleira P, Pereira P, Videira e Castro I

SESSION 2: Microbial Inoculants for Agriculture and Forestry

S2-P-01 "Evaluation of *Gracilaria gracilis* effects on tomato plants: pipelining the use of a new alga extract as biostimulant"
Rodrigues dos Santos AS, Santos AP, Vílchez JI

S2-P-02 "Effect of bacterial inoculation on the physiological response of strawberry under phosphorus deficit"
Mesa-Marín J, Valle-Romero P, García-López JV, Flores-Duarte NJ, Romano E, Redondo-Gómez S, Rodríguez-Llorente ID, Pajuelo E, Mateos-Naranjo E

S2-P-03 "Agronomic and environmental performance of rhizobial inoculants in common beans in the Dominican Republic"
Araujo J, **Urbano B**, González-Andrés F

S2-P-04 "And what is going on with MPB in real field situations?"
Barquero M, Crespo A, Ortíz-Liébana N, Mazuecos I, **González-Andrés F**

S2-P-05 "Physiological and genetic modifications in tomato plants subjected to moderate water stress and elicited with two plant growth promoting bacteria (PGPB)"
Lucas JA, Garcia-Villaraco A, Montero-Palmero MB, Montalban B, Gutierrez-Mañero FJ

S2-P-06 "*Bacillus* H47 activates DOXP and shikimate pathways modifying olive leaf antihypertensive activity under water stress"
Galicia E, Garcia-Villaraco A, Montero-Palmero MB, Gutierrez-Mañero FJ, Ramos-Solano B

S2-P-07 "Maize crops under rising temperatures: the effects of plant growth promotion rhizobacteria"
Pinto R, Bedia C, Cardoso P, Figueira E

S2-P-08 "Development of a biofertilizer based on biochar and PGPR bacteria in combination with compost"
Crespo-Barreiro A, Mazuecos-Aguilera I, Barquero M, Ortiz-Liébana N, González-Andrés F, Cara-Jiménez J

S2-P-09 "Effects of PGPB inoculation on the soil microbiome"
Carro L, Anza M, Saati-Santamaría Z, Blanco F, Garbisu C

S2-P-10 "Encapsulation of rhizobacteria and bacterial volatiles as biostimulants: application in the promotion of plant growth"
Duque B, Figueira E, Cardoso P

S2-P-11 "Improvement of agronomic performance and fruit quality parameters with an organic fertilizer amended with *Bacillus* sp."
Ortiz-Liébana N, Barquero M, Zotti M, Crespo-Barreiro A, Mazuecos-Aguilera I, **González-Andrés F**

S2-P-12 "Alfalfa-*Sinorizobium* rhizospheric communication in adverse thermal conditions"
Paulucci N, Cesari A, Nieva Muratore L, Castilla Marín V, **Dardanelli M**

S2-P-13 "Innovative inoculants: The use of legume seeds with optimized microbiomes"
Soares R, **Santos A**, Machado H, Barradas A, Fareleira P, Videira e Castro I

S2-P-14 "Selection of plant growth promoting bacteria for inoculation of pasture grasses"
Fareleira P, Santos A, Soares R, Machado H, Barradas A, Videira e Castro I

SESSION 3: Beneficial Microbes for Soil and Environment

S3-P-01 "Underestimated role of nodulated roots for soil fertility and quality"
Carranca C, Madeira M

S3-P-02 "Reduction in the use of herbicides favours nitrogen fixation efficiency in legumes"
Paniagua-López M, Jiménez-Pelayo C, Gómez-Fernández GO, García-Romera I, López-Gómez M, **Herrera-Cervera JA**

S3-P-03 "Can bacterial strains fight plant pathogenic fungi? Bacteria as biocontrol agents"
Matos D, Cardoso P, Molinero-Ruiz L, Figueira E

S3-P-04 "Response of soil microbiological activity and plant-beneficial microorganisms to the introduction of cover crops in intensive horticultural production systems"
Varela A, Soares R, Pereira P, Barradas A, Nunes AP, Videira e Castro I, Fareleira P

S3-P-05 "Metal tolerance and accumulation in white lupin (*Lupinus albus*)"
Vázquez-Rangel A, Martínez-Ballano P, Lucas MM, Pueyo JJ, **Quiñones MA**

S3-P-06 "Gene duplication and enhanced expression enable rhizobia tolerance in mercury-contaminated soils"
López-Pérez J, Clear M, Bhat A, Msaddak A, Epstein B, Tiffin P, Lucas MM, Paape T, **Pueyo JJ**

S3-P-07 "Characterization of leguminous root nodule microbiome"
de Castro Silva M, Machado H, Videira e Castro I

S3-P-08 "Screening for *in vitro* antifungal activity of plant beneficial rhizosphere bacteria against important forest pathogenic fungi"
Silva AC, Soares R, Videira e Castro I, Bragança H

S3-P-09 "Nematicidal potential of genus *Bacillus* against the root-lesion nematode *Pratylenchus penetrans*"
Costa M, Monteiro T, Barbosa P, Menéndez E, Espada M, Vicente CSL

SESSION 4: Nitrogen-Fixing Systems

S4-P-01 "Iron homeostasis in nitrogen fixation: Role of glutaredoxin5"
Rosa-Núñez E, Rubio LM, González-Guerrero M

S4-P-02 "*Azotobacter vinelandii* scaffold protein NifU transfers iron to NifQ as part of the iron-molybdenum cofactor biosynthesis pathway for nitrogenase"
Barahona E, Jiang X, Jiménez-Vicente E, Rubio L, **González-Guerrero M**

S4-P-03 "Role of MtYSL3 and MtYSL7 in symbiotic nitrogen fixation"
Castro-Rodríguez R, **Escudero V**, Abreu I, Imperial J, González-Guerrero M

S4-P-04 "Symbiotic characterization of chickpea germplasm and seed composition"
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S4-P-05 "*Sinorhizobium fredii* HH103 effectively nodulates *Robinia pseudoacacia*, a legume tree able to form indeterminate nodules"
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Vázquez-Arias D, Civantos C, Durán D, Bernal P, **Rivilla R**, Martín M

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S6-P-17 "Dissecting the role of extracellular membrane vesicles in the molecular dialogue between plant host and rhizobial strains"
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KEYNOTE CONFERENCES

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Prokaryotes constitute the oldest life forms on earth and represent true living fossils who help the reconstruction of the most ancestral processes of the biologic evolution. Prokaryotes are found as planktonic bacteria as well as cells intimately associated with other life forms—including plants—where the two constitute a holobiont. Both the numerosness of prokaryotes and their ability to horizontally exchange genes (*i. e.*, the capability of sharing previous experiences) has proven to be an efficient strategy for loading the bacterial pangenomes with redundancy, thus minimizing the loss of information and maximizing cell diversity and survival. By using such communal architecture, prokaryotes support an extraordinary collection of adaptive responses, consistent with the remarkably high ratio of their environment-exposed surface to their cellular volume. In such a general evolutionary context, plant-associated soil bacteria have accommodated—and in some instances expanded—their genomes in order to live under such contrasting and challenging circumstances as those represented by the oligotrophic soil and the particularly carbon-rich plant niche.

In this presentation I will focus on a few selected topics pointing to the way we have studied adaptive responses in soil bacteria covering specific instances of both their "free" and their associative life-styles. I will also discuss more general and basic forms of adaptation such as those involving the improvement of the genetic language.

In order to investigate adaptive processes in a reference soil alpha-proteobacterium we studied: (a) how cells responded to a model abiotic stress (acidity) and to the associated changes, and (b) how bacteria faced the challenge represented by the presence of rhizospheres, an ecologic paradigm of nutrient abundance for which bacteria from the bulk soil compete with each other. Our multiomic and functional studies revealed the central pathways that were necessary for improving bacterial fitness under low pH (Draghi et al., 2016), suggesting also the existence of a possible associative learning connecting the low pH signal in batch cultures to the expression of a more proficient plant invasion (Draghi et al., 2010). Bacteria thus appeared to be prepared not only to face the low pH *per se* but also to anticipate a response to circumstances that were likely to occur, such as a decrease in the number of roots and their responses to bacteria at low pH—indeed, anticipation is the minimal concept of learning. We do not yet know whether changes in the increased ability of acid-adapted bacteria to infect plants are derived from an improved capability of colonizing the rhizosphere, of penetrating root tissues more efficiently, or both. Independent experiments involving a phenomic approach demonstrated that the colonization of plant roots in our model alpha-proteobacterium was influenced by *ca*. 2% of the genome (more than 140 genes), thus pointing to the relevance of the bacterial interaction with plants (Salas et al., 2017). The chromosomal location of most of these genes highlighted the ancestral character of the bacterial approach to roots, with some novel traits expressing preferential colonization of specific rhizospheres having emerged more recently, that time being likely *ca*. 30 million years ago in the linage that we studied (fewer than 10% of the genes that we found affected specifically the colonization of particular plant rhizospheres as opposed to others).

Besides all these specific responses to environmental signals, we studied other more general adaptive strategy related to the way the language of the genetic code is used in bacteria. Languages are communication systems—either natural or formally created—that aim at the transmission of information between two physical and/or biologic entities: *i. e.*, languages are systems for the transfer of meaningful data. While spoken communication among humans has been the most thoroughly studied natural language, the genetic-code–based transmission of information constitutes, by far, the most ancient and ubiquitous natural language, and one that is also common (almost universal) and essential to all life forms as well as to viruses. This circumstance, and the early observation that cells do not make random use of codons with isoacceptor tRNAs, stimulated numerous investigations to understand the mutational and selective phenomena associated with the differential codon ("word") choices in organisms with remarkable differences in their global genomic compositions (GC contents spanning from less than 20% to *ca*. 80%).

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In order to investigate the trends and principles underlying specific codon preferences in the prokaryotic tree of life, we performed a comprehensive analysis of 29 different families within the domains Bacteria and Archaea and found 4 distinct behavioral groups (López et al., 2020).
The analysis of core-gene sets with increasing ancestries in each family lineage revealed that the codon usages became progressively more adapted to the tRNA pools. While, as previously reported, highly expressed genes exhibited the most optimized codon usage, the singletons always contained the less selectively favored codons. In agreement with previous reports, a C bias in 2- to 3-fold pyrimidine-ending codons, and a U bias in 4-fold codons occurred in all families, irrespective of the global genomic GC content. The U biases suggested that U_3-mRNA–U-tRNA interactions were responsible for a prominent codon optimization in both the most ancestral core and the highly expressed genes. A comparative analysis of sequences that encode conserved or variable translated products—with each one being under high and low expression levels—demonstrated that efficiency was more relevant (by a factor of 2) than was accuracy in the modelling of codon usage. Finally, after studying a model multipartite prokaryote genome, we performed a comprehensive analysis describing the inter- and intrareplicon heterogeneity of codon usages (López et al., 2019). Under the current view of the way cells make use of the 64 elements of their genetic code, novel parallels have to be elaborated to translate and contrast the classical definitions from cognitive language—like redundancy, synonymy (do fully synonymous codons exist?), ambiguity/polysemy (such as that associated with UGA codons) and contextual effects—all referring to different instances of plurality. That exercise will assist in understanding the minimal biologic needs and requirements that arose throughout evolution for the progressive emergence of specific semantic effects.

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Microorganisms are permanently challenged with hazardous environmental conditions that restrict their potential for survival and reproduction. To overcome this threatening many of them evolutionarily opted for a life in symbiosis. Fungi from the Glomeromycota phylum engaged in a life in mutualistic symbiosis with plant roots more than 450 million years ago. Since then, plants provide fungi with fixed carbon and in turn become an improved inorganic fertilization. Symbiotic arbuscular mycorrhizal (AM) fungi are major players in helping plants when growing under nutrient starvation conditions. They provide plants with phosphate and other nutrients and act as modulators of plant growth by changing the root developmental program during colonization. The establishment in the cortex of the hyperbranched hyphae of arbuscular mycorrhizal (AM) fungi called arbuscules is achieved by a massive reprogramming of the plant cell that allows to coordinate developmental changes with transport processes. This is only possible through a complex exchange of molecular cues between both partners that leads to the initiation of the symbiotic program. In the last years we have shown that fungal signals, of yet unknown nature, induce GRAS transcription factors that are recruited at arbuscule-containing cells to act in concert with DELLA proteins as positive or negative regulators of cortical cell size in *Medicago truncatula*[1,2]. From them, MIG1 outstands as a mycorrhiza-specific positive regulator, determining the shape and size of cortical root cells. MIG1 interacts and requires DELLA for its function at increasing cell size. Cell expansion in arbuscule-containing cells is restrained by two other GRAS transcription factors MIG3 and SCL3, that also act in concert with the central regulator DELLA and antagonize the function of the complex MIG1-DELLA. To investigate the possible fungal signals that might contribute to this reprogramming we have been focusing in the identification and characterization of AM effector proteins[3,4]. Our results have shown that some of them are able to induce developmental changes when expressed in planta and thus could be responsible for at least part of the plant cell reprogramming occurring during symbiosis. On how plant and fungi talk and react to each other to achieve an almost perfect relationship will be the focus of this talk.

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The amazing versatility of molecular tools used by rhizobia to switch between complex lifestyles

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Scientists have been working for years to understand the molecular mechanisms governing the nitrogen-fixing symbiosis of microorganisms with legume plants to improve its efficiency and hoping that, in the future, this trait could be transferred to other plant crops. However, certain issues regarding the complex molecular dialogue between bacteria and its cognate plant host remain unknown. Bacteria are capable of thriving in diverse and changing biotic and abiotic niches thanks to their physiological plasticity and adaptive responses to environmental cues. Rhizobial transition from soil to host-dependent conditions demands a tightly and coordinated regulation of the expression of key genes upon perception of a variety of signals. For example, characterization of the first steps of the rhizobia interaction with legumes uncovered an extraordinarily versatile single hydrolytic enzyme serving several functions during nodulation (i.e. adhesion and biofilm formation, signaling and plant primary and secondary infection; reviewed in 1).

Among the diverse means of bacterial fine-tuning of gene expression, small non-coding RNAs (sRNAs) that bind to proteins or base pair with target mRNAs have been recently screened in rhizobia. It has become clear that sRNAs have key roles in prokaryotic physiology by regulating important biological cell processes. As expected, several sRNAs are involved in rhizobial adaptation and survival in changing environments, even during their symbiotic interaction with plants. Furthermore, the first prokaryotic sRNAs involved in essential functions as modulation of cell cycle progression and bacterial division under stress conditions (EcpR1 and GspR1) where described in rhizobia (reviewed in 2). Novel sRNA-biding proteins (MetK) and unprecedented catalytic features of enzymes that assist RNA regulation, like the unique ribonuclease YbeY, have been first uncovered also in rhizobia (reviewed in 3). Deciphering the role of sRNAs and the binding proteins that control rhizobial stress adaptation and survival in the soil and nodule environments, probably through *trans*-kingdom RNA regulation, will open the field to further biotechnological applications.

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SESSION 1

Diversity and Ecology of Plant Beneficial Microbes

S1-L-01
Call-for-help, a potential microbe-shaping biotech in agriculture: from beneficial diversity to soil health recovering

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Agriculture is at the gates of a new and necessary revolution. The pressure caused by the depletion of the soil, as well as by the increasingly harsh climatic conditions, are leading to the search for alternatives for fertilization and improvement of cultivation methods. In this scenario, the microbiota is playing a very relevant role. Since the mid-1970s, the enormous potential of using microorganisms as fertilizers, stress tolerance enhancers or as pest and pathogen biocontroller, has been frequently reported. However, it has been more recently when, through the use of massive sequencing techniques and precision analysis, we have been able to generate complex networks of these beneficial interactions. Microbial diversity has provided with very valuable information regarding what species, population relevance or changes occur throughout ecosystem dynamics. This opens the door to the use of bioengineering techniques to improve soil health, microbial biodiversity or patterns of interactions. Among them, the use of root exudates to anticipate the presence of beneficial microbial communities, the composition of synthetic communities (SynComs) or the shaping of agricultural soil communities by implementing the transfer of stress-adapted beneficial microbes stand out. In this context, we will introduce the results of work on the stress-root exudate-recruitment relationship [1], and the use of drought-adapted wild legume microbiota as part of the LegumBiome project, as case studies [2].

In this last work, we aim to describe the cultivable communities associated with wild legumes, adapted to desert conditions with the aim of characterizing candidate strains to improve the response of plants to drought. To do this, we are carrying out sampling in different parts of the Eastern Spain, corresponding with those regions reported as under risk of desertification, including Navarra, Huesca, Zaragoza, Soria, Madrid, Albacete, Jaén, Granada, Almería, Murcia and Alacant. Collected samples included rhizosphere soil, roots, nodules and seeds from herbaceous and shrubby species alive during the summer months. Culturable strains from these samples were identified and characterized based on their xerotolerance, production of phytohormones related to drought response, and colonization skills. Those with the best results are being tested on commercial crop legumes (*Phaseolus vulgaris*, *Len culinaris*, *Cicer arietinum*) to assess their drought-tolerance enhancement, as well as their production rate under such conditions. So far, we have isolated more than 400 strains from up to 25 different plant species. Among them, about 20 strains have shown to be effective to enhance the response of plants to drought. The molecular and metabolomic characterization of these interactions will allow us to better understand the mechanisms used, as well as the possibility of using such strains as bioinoculants capable of improving crop yields under more severe conditions of water deficit.

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S1-O-01
Wheat-canola root-associated bacteriomes reveal common and unique taxa with diverse behaviours when combined into synthetic bacterial communities

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Plants live in close association with their microbiomes, which might be subjected to management strategies, boosting the presence of functionally relevant bacterial taxa. These strategies will improve the plant growth and development and enhance the yields of crops. The selection of agronomic practices and water and fertilization regimes is also very important to maximize crop productivity. In this sense, wheat-canola crop rotations are quite common worldwide; however, the studies on those crops' microbiomes are more focused in wheat and in both crops as individuals rather than in rotation (Schlatter et al 2019). Canola is an agroeconomic valuable crop widely studied to improve yields and which root microbiota have been studied under culture dependent/independent approaches (Lay et al 2018; Jiménez-Gómez et al 2020). The main aim of this work is to determine the core bacteriome and their associated functions that are common between canola crops preceded by wheat grown in two soils with different water regimes: rainfed and irrigated. Using a multidisciplinary approach, we determined the core bacteriome of both fields and found common and unique features. The most abundant taxa belonged to the phylum Proteobacteria, order Rhizobiales, and the phylum Actinobacteria, orders Rubrobacterales and Micrococcales. It is noteworthy the abundance of the archaea from family Nitrososphaeraceae. The analyses revealed that the genera *Rubrobacter* and *Pseudoarthrobacter* showed strong association with rainfed canola and the family Nitrosomonadaceae and also, the genus *Ensifer* with irrigated canola soils. Moreover, we isolated, identified, and functionally characterized a collection of canola root-associated strains from both fields. Combining strains according to core, abundancies, root association level and plant growth promotion features, we design seven SynComs and tested in wheat and canola under two levels of fertilization. Best-performing SynComs were the ones designed with PGP rhizospheric/endospheric strains when inoculated on wheat, the preceding crop. Remarkably, the development and growth of canola plants inoculated and treated with reduced level of fertilization were improved, independently of the inoculated SynCom. Overall, the knowledge and application of native bacteriomes will set up the basis for designing effective bacterial consortia to improve productivity and yield of wheat-canola rotations in cropping systems under sub-optimal conditions.

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S1-O-02
Cover crops promote beneficial soil microbiomes but have limited impacts on soil nutrient cycling in citrus orchards

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The influence of cover crops (CCs) on soil nutrient cycling, citrus production, the abundance and diversity of nitrogen (N)-cycling microbial communities, and the diversity and composition of bacterial and fungal communities in tree crops has been poorly explored (Castellano and Strauss 2020). We examined the effect of replacing the traditional weedy inter-row middle of two commercial citrus orchards (COA and COB, respectively) in Florida with two different mixtures of CCs: legumes and non-legumes (LG + NL) and non-legumes only (NL). A no-treatment/grower standard was used as a control (GSC). Soil samples were taken from the treated row middles prior to the experiment start and after three years. After that time, the use of both CC mixtures significantly increased soil C availability in the row middles of COA but had no significant effects on soil C content in COB. Significant increases in ammonium availability and the abundance of nitrification genes were observed in soils treated with LG + NL in COA, suggesting that biological N-fixation contributed to improved N availability. CCs had no effects on ammonium content in COB. No significant differences in nitrate content were detected between treatments in row middles of both citrus orchards. However, treatment with LG + NL significantly increased the abundance of denitrification genes (*nirK* and *nosZ*I) in COA compared to NL and the GSC. Regardless of the citrus orchard, CCs had no significant effect on citrus production after 3 years. Treatment with LG + NL and NL significantly increased the diversity (number of taxa and values of the Shannon index) of bacterial and fungal communities compared to GSC in COA after 3 years but had no impact on the soil microbiome in COB. Planting both mixtures of CCs significantly altered the composition of the bacterial and fungal communities in COA. Specific bacterial and fungal genera (some of them associated to plant-growth promoting bacteria such as *Bacillus*, *Rhizobium*, *Allorhizobium*, *Pseudomonas*, *Glomus*, Erwinia, and *Actinomyces*) were identified as potential indicators for changes in bacterial and fungal communities after planting CCs in COA and were treatment-specific, suggesting that CC mixture differentially drives changes in the soil microbiome. We conclude that: 1) CCs can improve soil nutrient cycling but may have limited impacts depending on the location likely due to differences in soil properties, environmental conditions (e.g., temperature and precipitation, and citrus management); 2) CCs drive changes in the abundance of N-cycling communities linked to soil nutrient cycling and impact the soil microbiome promoting the proliferation of potential beneficial taxa for plant growth. Whether increases in soil C and N availability could eventually be translated to improved citrus production should be further explored as it may take several years to observe increases in tree productivity as citrus fruit takes nearly a year to mature.

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S1-O-03
The role of soil microbiota and environmental cues in the regulation of maize development

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As a result of climate change, the amount of greenhouse gases in the atmosphere is increasing, temperatures are rising, precipitation patterns are modified, and extreme weather events are occurring more frequently. These changes accounted for 25% of average yield losses for low-latitude maize. To tackle the reduction of productivity, several strategies have been developed, such as altering planting and harvesting time, sowing crops with a short life cycle, applying crop rotation and irrigation techniques, and introducing variation in cropping systems. Further to these strategies, a knowledge-driven approach to managing the soil microbiota rather than merely applying microorganisms has been described as a promising approach to sustainably increase crop yield. Taking this into consideration, we have designed a holistic approach to understand how plant mechanisms and microbiota coordinate to respond to an environmental stressor in the model plant *Zea mays* (maize). This approach combines the characterization of (1) a collection of agricultural soils, (2) soil bacterial composition, (3) plant microbiota, and (4) water and nutrition availability. We monitored free-living soil bacterial communities, soil mineral content, and climatic conditions in 24 agricultural soils in the Sahel region (Cabo Verde) for two years. We observed strong structuring of the free-living bacteria according to the soil mineral content, but unrelated to climatic conditions. Since plants recruit their symbionts from the nearby free-living microbes, we have further evaluated the factors driving microbiota assembly in root and maize single leaves, in three different soils. We found that individual maize leaves harbour distinct ionome and microbiota compositions. Then we designed a synthetic community (SynCom) to evaluate bacterial effects on single leaf growth and we found that leaves respond differentially to the SynCom by suppressing genes on the ABA pathway. Collectively, our work will help to understand the biotic and abiotic cues in the regulation of maize development and will help design microbial synthetic communities with a predictable effect on plant host. This will support the development of more efficient and environmentally-friendly agricultural practices.

S1-O-04
Tailoring the cultivable bacterial microbiota as a source of stage-specific biofertilizers

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World population is increasing and a major pressure on food production is expected. The present agricultural methodologies are vulnerable to the erratic climate, increasing urbanization, industrialization, and agrochemical pollution. To achieve food security, sustainable practices must be pursued. The common bean (*Phaseolus vulgaris L.*) is one of the most produced legumes worldwide and, as a reliable source of high-quality protein, can reduce the environmental impact of meat production. However, high yields are dependent on heavy and expensive fertilization. Plant growth promoting rhizobacteria (PGPR) are emerging as a sustainable prospect to increase agricultural production, yet this interaction is not fully understood, especially the chronological variations in the microbiota. Thus, a deeper understanding on the interaction and dynamics between plants and microorganisms may boost the beneficial effects of microorganisms on plants. To reach this goal, the cultivable microbiota of the bean root was isolated and identified at distinct stages of plant development (early vegetative growth (V1), late vegetative growth (V2), flowering (F), and pod (P)) and root compartments (rhizoplane (out), endosphere (in), and nodules (nod)). Diversity and abundance of cultivable bacteria associated to root compartments differed throughout plant life cycle. Bacterial plant growth promotion and protection abilities (indole-3-acetic acid production, siderophores synthesis, and antifungal activity) were determined and associated to the plant phenology, suggesting that among the cultivable bacteria associated to the plant root several strains had an active role on the response to plant biological necessities at each development stage. Several strains stood out for their ability to display one or more plant growth promoting (PGP) traits, being excellent candidates as efficient stage-specific biostimulants to be applied in precision agriculture.

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S1-O-05
Biotechnological potential of *Blastococcus* (Actinobacteria) in arid agro-ecosystems

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The genus *Blastococcus* (*Geodermatophilaceae, Actinobacteria)* comprises ten species isolated from sediments, desert soil, decaying monuments and plant leaves. Studies based on genomic data are still scarce. In particular, just two papers, focused on single strains, were published so far, which revealed numerous genes involved in stress response and adaptation to harsh habitats apart from an exceptional potential to produce novel natural compounds. In this study, we aim to taxonomically characterise four novel species within the genus *Blastococcus* and explore their biotechnological potential to be applied in arid agro-ecosystems. Our results indicate that *Blastococcus* genomes encode a variety of mechanisms involved in adaptation to environmental stress and a high versatility in carbon metabolism and extracellular enzymes. It is suggested that *Blastococcus* could have an unexpected and remarkable role in soil carbon fixation but also in the decay of organic residues, transformation of native soil organic matter and mineralisation of nutrients available for plants. The metabolism of *Blastococcus* could therefore be key to the natural restoration of soil fertility in arid and degraded soils.

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S1-O-06
Diversity of root nodule bacteria associated with *Spartocytisus supranubius* across Teide National Park soils

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Spartocytisus supranubius (Teide broom) is the most representative legume in the Canarian high mountain ecosystem. This legume plays a pivotal role, since the symbiosis it establishes with rhizobia has proven to be the main input of nitrogen in this otherwise nutrient-poor environment (Wheeler & Dickson, 1990; Pulido-Suaréz et al., 2021). Despite its importance, little research has been done on the characterization of this symbiosis.
In the present study, we sampled rhizospheric and non-rhizospheric soils in several areas of Teide National Park with different biotic and abiotic stresses and recovered over 100 strains from root nodules of *S. supranubius*. We aimed to answer: How diverse are the root nodule bacteria of *S. supranubius*? Does the rhizobia nodulating *S. supranubius* vary through the Park soils? If so, which are the main factors affecting the genotypes' distribution?
The isolated bacteria were characterized by sequencing and the physicochemical properties of the soils were also analyzed. Principal component analysis was performed to find out whether soil characteristics could affect bacterial distribution.
Results showed that *Bradyrhizobium* is the main microsymbiont of broom plants, in accordance with previous studies (Pulido-Suárez et al., 2021). However, within this genus, the isolates clustered in three main clades corresponding to different species. Interestingly, differences on the species distribution were noted along the locations. In addition, non-nodulating bacterial endophytes were also isolated from the root nodules, *Agrobacterium* being the most abundant.

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S1-P-01
LegumBiome-Drought Project: Screening of potential plant drought-tolerance enhancers among wild, desert-adapted legumes

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Current climatic conditions tend to make stressful phenomena for agricultural production more intense and frequent, such as periods of drought, high temperatures or radiation received. In this scenario, losses are expected to be around 10-25% on average [1]. For some crops, such as legumes, this situation can be aggravated by their special sensitivity to lack of water, where such losses are estimated to reach up to 60% [2]. Legumes represent one of the most nutritionally complete crops, being a staple food in many regions of the world. In this way, the cultivation of these plants can be strongly conditioned in rainfed regions. Interestingly, a possible solution would come from these areas, since we can find legumes naturally adapted to arid and semi-arid environments. They become particularly relevant for the kind of interactions they perfome with the local microbiota. It is well known that legumes can interact with different species and strains of *Rhizobium*, however, more and more microbiota capable of interacting under stressful conditions are being reported, becoming very relevant for the development of new stress-tolerance treatments [3].

Thus, in this work we aim to describe the cultivable communities associated with wild legumes, adapted to desert conditions with the aim of characterizing candidate strains to improve the response of plants to drought. To do this, we are carrying out sampling in different parts of the southeast of Spain, one of the driest regions in the country and most affected by desertification, including Jaén, Granada, Almería and Murcia. These species were sampled from the rhizosphere soil and the roots of herbaceous and shrubby species during the summer months, and were identified and characterized based on their xerotolerance, production of phytohormones related to drought response, and the production of protective compounds. Those with the best results are being tested on commercially harvested plants (*Phaseolus vulgaris*, *Len culinaris*, *Cicer arietinum*) to assess their improvement in response to severe water deficits, as well as in terms of production under such conditions. So far, we have isolated more than 400 strains from up to 25 different types of plants, obtaining some 20 strains that have shown notable improvements in the response of commercial plants to drought. The molecular and metabolomic characterization of these interactions will allow us to better understand the mechanisms used, as well as the possibility of using such strains as bioinoculants capable of improving crop yields under more severe conditions of water deficit

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S1-P-02
Evaluation of the microbiota associated with a salt-tolerant rice variety as a treatment to induce resistance in sensitive varieties

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During this century, the world demographic pressure has risen sharply. In just one generation, the world population has tripled, requiring similar rising in agricultural production. Rice (*Oryza sativa* L.) is one of the most important crops worldwide, feeding almost half of the global population. However, as a result of climate change crops are highly submitted to abiotic stress conditions. Among them, soil salinity is considered as one of the major problems for agriculture, impacting not only the plant itself but also the soil and living organisms surrounding. Rice is considered salt sensitive by nature and when subjected to salt stress, it suffers an enormous negative impact in metabolic mechanisms, growth and productivity. Plant growth-promoting bacteria (PGPB) are able to promote plant vigor and improve adaptation in stress conditions, by producing phytohormones, secondary compounds, osmolytes and antioxidant enzymes[1]. The exploitation and identification of salt-tolerant plant growth-promoting bacteria (ST-PGPB) may be the key for the development of novel mechanisms to coup with salt stress resistance. Several strains have been reported to successfully improve plant growth attributes, soil enzyme activities, microbial counts, and mitigating the deleterious effects of salinity in rice (osmoprotectants, compatible solutes)[2,3]. Our work aims to evaluate the effect of ST-PGPB candidates selected from Pokkali (salt-tolerant rice variety) associated microbiota (seedborne and root recruited) in the level of salt stress resistance and responses of IR29 and Nipponbare (salt sensitive varieties). Initially, the culturable endophytic microbiota of all rice varieties was isolated and genetically identified to evaluate population patterns and discern best candidates solely present in the salt-resistant rice variety. Then, a total of eight strains were selected and screened by their skills and growth rate in salt at different concentrations (up to 2 M), including biofilm formation, auxins, ACC deaminase production or exopolysaccharides (EPS) production. Three of the eight tested strains were selected (*Bacillus altitudinis, Bacillus subtilis, Lysinibacillus fusiformis, Paenibacillus pabuli*), presenting always better performance at 500 mM compared with the control conditions. The effects of these strains in the physiological development of the sensitive rice varieties were tested under non-saline and saline hydroponic conditions, evaluating positive changes in their survival rate, phenotype, growth and developmental timing. Moreover, a targeted DNA methylation detection technique (CHOP-PCR) was used in order to understand the role of the candidate strains during the salt stress response process, and the involvement of main epigenetic marks of the process. These candidates will serve as bioinoculant treatment to amend the rice performance (particularly salt-sensitive varieties) under saline stress conditions, improving survival rates and production levels under such conditions.

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S1-P-03
The rhizosphere microbiome associated with the legume *Spartocytisus supranubius* in the high mountain ecosystem of Teide N.P.

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Spartocytisus supranubius (the 'Teide broom') is the dominant and most characteristic legume in the shrubby vegetation of the high-mountain ecosystem of Teide National Park. In this ecosystem, the rhizosphere microbiota must be a key factor for plants to cope with the harsh conditions causing various abiotic stresses. We have used high-throughput sequencing to characterize the bacterial and fungal rhizospheric microbiomes of broom plants.
The results showed bacterial communities with higher richness (ASV), diversity and evenness compared to fungal communities. The composition of the bacterial microbiome was characterized by a community in which the phyla Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Bacteroidetes accounted for about 75% of the relative abundance. Verrucomicrobia, Gemmatimonadetes and Firmicutes together with other minority phyla account for another 20% and 8% of unclassified phyla complete the microbiome. At the genus level, the highest abundance was for the Chloroflexi *Thermogemmatispora*, the Actinobacteria *Solirubrobacter*, *Crossiella* and *Mycobacterium*, the Acidobacteria Candidatus *Solibacter*, the Gemmatimonadetes *Gemmatimonas,* while *Bradyrhizobium* was the most abundant Proteobacteria, which is not surprising as bradyrhizobial species are nitrogen-fixing microsymbionts of Teide brooms.
The fungal microbiome is dominated by phyla Ascomycota and Basidiomycota, which make up 80% of the relative abundance of the community. Mortierellomycota is the third most abundant phylum and contains the most abundant fungal genus, *Mortierella*. The Glomeromycota, the phylum containing arbuscular mycorrhiza (AM), account for 1% of the relative abundance.
Apart from the clear benefits expected from symbioses with bradyrhizobia and AM, *Mortierella* have been also associated with plant growth promotion. The role of the other microorganisms in this legume rhizosphere and whether they have plant promoting activities is not yet known, and should be addressed in future research.

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S1-P-04
Trichoderma species and arbuscular mycorrhizal populations associated to the banana rhizosphere affected by *Fusarium oxysporum* f. sp. *cubense* in the Canary Islands

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Bananas are one of the most important crops in the Canary Islands, with more than 9,000 ha under cultivation. Several diseases affect this crop, among them: *Fusarium* wilt of banana, also known as Panama disease, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Currently, there is no effective method to control this disease and, in this context, native soil microorganisms, such as genus *Trichoderma* and mycorrhizal fungi, play a major role in protecting the plant against the pathogen. The aim of this study was to analyse the population of mycorrhizal fungi and *Trichoderma* in the rhizosphere of banana plants with and without Panama disease symptoms. Fourteen banana farms located in different bioclimatic zones of the island of Tenerife were analysed. On each area, rhizospheric soil samples were collected from three symptomatic and three asymptomatic plants. The following determinations were carried out on each soil sample: a) Isolation and identification of *Trichoderma* species; b) Quantification of the number of arbuscular mycorrhizal (AM) chlamydospores; c) Mycorrhizal colonization in roots of banana plants developed under controlled conditions. A total of 210 *Trichoderma* isolates were obtained (100 and 110 samples of soil from symptomatic and asymptomatic plants, respectively). Twelve species were identified: *Trichoderma gamsi, T. hirsutum, T. cf. lixii, T. harzianum, T. cf. harzianum, T. virens, T. guizhouense, T. atrobrunneum, T. asperellum, T. hamatum, T. afroharzianum* and *T. longibrachiatum*. The predominant species were *T. cf. harzianum* and *T. virens*, in both sample types (rhizosphere of symptomatic and asymptomatic plants). Resuls of micorrhizae have a high variability depend on the sampling area (north or south of the island) and the health status of the In most cases, no correlations were observed between the number of *Trichoderma* species and the variables related to the quantification of mycorrhizae. However, in the northern part of the island, a positive correlation was observed between the number of *Trichoderma* species and the percentage of mycorrhizal colonization in roots of plants with and without Panama disease symptoms. Significant differences in the percentage of mycorrhizal colonization were detected between soils from symptomatic and asymptomatic plants from the southern zone. The results of this work show the population distribution of potentially beneficial microorganisms in presence of *Foc*, which might increase the knowledge of the interactions between them, helping in the desing to biologial control strategies.

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S1-P-05
Biodiversity of rhizobia-nodulating lentils in Spanish soils

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Lentil (*Lens culinaris* Medikus subsp. culinaris) is one of the oldest crops that was domesticated in the Fertile Crescent around 9,000 years ago (Toklu et al., 2009). The region of origin encompasses Southeastern Turkey and Northern Syria and, via Danube, spread to Europe. Today, it is an important food legume crop in the farming (soil fertility management) and food systems of many countries globally. According to FAO, the global production of lentils reached 6,537 thousand metric tons in 2020 being Canada and India the major producers. Nevertheless, little is known about the microsymbiont (rhizobia) which fix nitrogen in symbiosis with this crop. Traditionally, lentils have been considered to be nodulated only by *Rhizobium leguminosarum* bv viciae but, lately *R. laguerreae* or the new species *R. lentis*, *R. bangladeshense* and *R. binae* (Taha et al., 2017 and Rashid et al., 2015, respectively) have been isolated from lentils nodules. However, the new species were not able to form effective nodules on lentils under laboratory conditions.

To address the rhizobial population associated to lentils in Spain, an assay for studying both, the population and the biodiversity of rhizobia-nodulating lentils, has been carried out with soils coming from two different local sites: experimental farm of Albaladejito (Cuenca) and Carmona (Sevilla), with different histories of lentils cultivation (recent record and no records, respectively).

The results obtained after the inoculation of lentils (cv Pardina) with these soils by the NMP technique, showed that the number of these rhizobia were higher in Albaladejito than in Carmona (5,8 x 10^5 vs 1,1 x 10^3 rh/g soil). A total of 54 rhizobial strain were isolated from nodules of this legume, 32 from lentils inoculated with soil of Carmona and 22 from lentils inoculated with Cuenca soil. After a first ERIC PCR screening for removing duplicities, a total of 12 (Carmona) and 20 (Cuenca) unique isolates were obtained. The 16sRNA gene sequence clustered all the isolates together with *R leguminosarum*. The subsequent phylogenetic analysis of housekeeping genes (*atpD, glnII and recA*) allowed a better discrimination, clustering the isolates with *R. laguerreae* and, for the first time until we know, with the species *R changzhiense*. Several isolates remained with no clear affiliation and could represent new species.

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S1-P-06
Isolation and identification of rhizobia from cowpea nodules *Vigna unguiculata* (L.) Walp. grown on the Peruvian coast

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The cowpea, *Vigna unguiculata* (L.) Walp, establishes atmospheric nitrogen-fixing symbiosis with soil bacteria, called rhizobia (Valdez et al. 2016). In recent years, the productivity and profitability of cowpeas in Peru has been affected by various factors, highlighting soil degradation, and being aggravated by the nitrogen fertilizer crisis (FAOSTAT, 2022). Rhizobial inoculants are a sustainable alternative to nitrogen fertilizers, however, little is known about the diversity and identity of rhizobia associated with cowpea nodules that allow the design of inoculants. The objective of the research was the isolation, characterization and identification of rhizobia strains associated with cowpea nodules. Four collections of cowpea nodules were made in the Piura region (02) and Lima region (02). Each nodule was sterilized and streaked onto YEM (Mannitol Yeast Extract) Agar and incubated at 28°C ± 1 for 20 days (Hungria et al. 2016). 103 isolates were purified and preserved in glycerol at 30% (v/v) at -20°C. For authentication, isolates were grown in YEM broth and inoculated on seeds of cowpea var. "vaina verde" growing in plastic growth pouches, at a rate of 1 mL per seed, for 21 days. Only 65 strains were authentic rhizobia (63.1%), of which only 50.8% (33 strains) presented moderate to adequate effective nodulation. For genomic DNA extraction, strains were cultured in 3 mL of TY broth and incubated at 28 ± 1°C at 150 rpm for 3 to 4 days. 32 genomic profiles (BOX-PCR) were formed, with a greater distribution in strains from Lima (18) compared to strains from Piura (13). The 16S rRNA phylogeny indicates that all 32 strains belong to the genus *Bradyrhizobium*. The results show the high promiscuity of cowpea in its rhizobial requirements, reflecting at the morphocolonial, nodulation and molecular levels, evidencing the opportunity to select elite strains of nitrogen-fixing rhizobia.

Key words: *Bradyrhizobium*; BOX-PCR; Nodulation; Genetic Diversity; 16S rDNA.

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S1-P-07
Microbiome analysis of wild blackberry and blueberry plants in the northern Iberian Peninsula

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The constant co-evolution of microbes and plants led to precise and beneficial specific inter-kingdom interactions. The evolutional success in these interactions through millions of years led to wild plants to adapt in their native environments by taking advantage of their symbionts, and vice versa. However, it has been demonstrated the use of intense agricultural practices have important effects on the soil and plant microbiome composition, something that can affects the plant health status. Thus, the study of wild plant microbiomes can be beneficial to design efficient biofertilizers that mimic the natural plant-microbial compositions, but for that, considering the spatial scaling is of utmost importance to draw correct conclusions and to find plant-microbe co-adaptation patterns.

Blueberries (*Vaccinium* spp.) and blackberries (*Rubus ulmifolius*) are considered functional foods with rich nutritional value; based on that, these berries are becoming increasingly popular among consumers. Despite their importance, the literature lacks studies on the microbial communities of blackberry plants, and the those on blueberries are scarce.

Here, we aim to decipher the microbial communities of both wild blueberry and blackberry plants along different forest ecosystems in the Iberian Peninsula. We analyzed 48 microbial communities (16S rRNA and ITS amplicons) from roots and rhizospheres.

Proteobacteria is the main taxa on both roots (45.8-76.8%) and in some rhizosphere samples (28.8-44.1%). Acidobacteria is the second phyla in relative abundance (12.8-41.2% on rhizospheres; 0.8-26.2% on roots), followed by Bacteroidetes and Actinobacteria**.** In contrast, the fungal communities are less consistent among the different samples' types and sample sites. For example, the abundance of Hyaloscyphaceae, which is the most abundant family, ranges from almost a 40% of the reads in some samples but is absent or almost absent in others. Here we found taxa that are considered as ericoid mycorrhiza, such as Helotiales (the most abundant order, mainly in blueberry, that is an erycoid plant), *Oidiodendron* sp., *Pezoloma ericae* o *Phialocephala* sp.

We also searched specific enrichments or exclusions of microbial taxa in the endospheric tissues to unravel the selective filter that differentiates the rhizospheric and the root microbiomes in these plants. Concretely, both blueberry and blackberry roots exclude significantly many diverse microbial taxa, but only 4 genera are significantly enriched in these roots, *Nevskia* in blueberry roots and *Novosphingobium*, *Sphingobium* and *Steroidobacter* in blackberry roots.

In sum, our data demonstrate that there is a similar occurrence pattern of the main fungal and bacterial taxa in blueberry and blackberry plants, although some taxa show specific enrichments in each plant species. Also, we presented the first insights into the microbiome composition of blackberries. These results will be useful to understand the microbiology of forests and, concretely, from these two specific plants. Finally, we believe that this research will serve as the basis to the development of new and efficient biofertilizers that target any or both blueberry and blackberry crops.

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S1-P-08
Following the rhizobia-legume symbiosis in the Montado ecosystem

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Montado ecosystem has a high socioeconomic value by combining exploitation of cork with livestock husbandry, pastures, among other added-value activities. In Portugal, Montado soils are often degraded, acidic, with high concentrations of aluminum and manganese, and with low overall fertility, which is an alarming situation for the sustainability of this ecosystem. One of the most important aspects for this ecosystem is the establishment and quality of pastures. Pastures play a critical role in soil sustainability by providing feedstock, soil conservation, water retention and nitrogen and CO_2 fixation. From these, pasture legumes greatly improve soil fertility by fixing nitrogen when in symbiosis with rhizobia bacteria. This symbiotic association is often highly specific with implications in plant growth promotion and ultimately in soil fertility.

In this context, this work aims to monitor the evolution of the rhizobia-legume associations in the Montado ecosystem subjected to a field trial under non-treated and treated conditions for soil correction. Understanding how legume-rhizobia association evolve over time upon different correction treatments is of utmost importance for having the most adequate management practices.

It was installed a field assay in a Montado area and different treatments were applied for soil correction (addition of dolomitic limestone and/or cellulosic sludges). Legume-rhizobia associations were monitored by the evaluation of the natural rhizobial population size, genetic diversity, taxonomic identification and strain symbiotic effectiveness (nitrogen fixation). Natural rhizobial population size was evaluated by the Most Probable Number method using *Trifolium subterraneum* and *T. resupinatum* as trap hosts. Genetic diversity of bacteria isolated from root nodules was assessed by REP-PCR fingerprinting. Non-redundant bacteria strains were selected from the REP-PCR clades and were taxonomically identified by 16 rRNA and *rec*A gene phylogenies.

Results showed the presence of high numbers of rhizobia bacteria with high genetic diversity despite the low soil quality. The natural population was also capable of fixing nitrogen at high rates when inoculated in *Trifolium* spp.. Genetic diversity of the rhizobial population increased overtime during the field trial. High symbiotic effectiveness was found in isolates from the root nodules of *Trifolium* spp. irrespective of the treatment and isolation year. Strains capable of establishing highly effective symbiotic association with *Trifollium* spp. were identified as *Rhizobium leguminosarum*.

In conclusion, in the Montado area studied a large natural rhizobial population existed with high genetic diversity that can be further exploited and improved by the practice of adequate soil management. Rhizobia-legume associations together with soil physical-chemical conditions are crucial for the sustainability of the Montado ecosystem.

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XVIII SEFIN OEIRAS '22

BeMiPlant

Beneficial Plant-Microbe Interactions

SESSION 2

Microbial Inoculants for Agriculture and Forestry

S2-L-01
Bacterial Synthetic communities (SynComs) as inoculants for agriculture and environmental protection

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Enrichment cultures are useful for the construction of bacterial consortia to be used as inoculants, both for plant growth promotion (PGP) and for bioremediation. However, these consortia frequently contain bacteria that are undesirable, including bacteria that do not participate in the biotechnological process or even bacteria that might be harmful or pathogenic for humans, animals and/or plants. Deconstruction of consortia allows the design of SynComs that contain the bacterial taxa necessary for the inoculant function and are free from undesirable populations. SynComs are therefore rationally designed, taking into account the principles of functional redundancy and phylogenetic diversity to optimize their performance. In the design and construction of SynComs, culturomics are used to isolate strains from the consortium, either by using different, selective growth media or by robotical isolation by dilution in a general, permissive medium. Isolated strains are tentatively identified by 16S DNA sequencing and the metagenome of the designed SynCom is then determined, in order to establish its biotechnology potential. Here we are presenting the design and construction of two SynComs, one designed for bioremediation of hydrocarbons and the other for PGP of tomato plants. For the first SynCom, soil polluted with heavy oils was used as the source of the enrichment consortium. This consortium was obtained after growth in diesel as the sole carbon and energy source. The consortium contained around fifty different taxons (Amplicon Sequence Variants, ASVs) including some undesirable bacteria such as Enterobacteria, *Acinetobacter spp*, *Stenotrophomonas spp.* among others. The final SynCom is composed by eight strains that harbour the same metabolic potential than the whole consortium. The second consortium was isolated from tomato plants rhizosphere, growing in a commercial plot. Root exudates and extracts were used as the sole carbon, energy and nitrogen sources. The consortium contained 75 ASVs, including some of them potentially harmful. A SynCom harbouring multiple PGP traits was designed and constructed. This SynCom has been tested on tomato plants and has shown to significantly improve the yield under greenhouse experiments.

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S2-L-02
Beneficial microbes for forestry applications

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Microbial biodiversity associated with the plant-soil system is an increasingly recognized effective resource in managing plant health and soil fertility (Gupta et al., 2022). It is now well accepted that the microbiome of plants has profound impacts on their survival and performance, helping them to overcome environmental challenges such as nutrient limitation, herbivore and pathogen attacks, or abiotic stresses. Plants usually establish symbiotic associations with mutualistic and/or free-living beneficial microorganisms, as a crucial nutrient-acquisition strategy to adapt to the environment, which may be of particular importance under the current global change scenario.

Significant improvements in economic and environmental sustainability of agriculture and forestry can be achieved though a better understanding of plant-microbial interactions (Cardon and Whitbeck, 2007). The use of native selected symbiotic microorganisms offers the possibility to efficiently use natural resources for improving forestry industry, mainly through promoting the physiological quality of seedling nursery stocks while reducing inputs such as fertilizers, water or chemicals, and ensuring the survival and fitness of trees after out-planting in the field. Beneficial microbes are increasingly being used for multiple applications in forestry and agro-forestry. The success of a given reforestation and/or afforestation project is tightly linked to the vigor and adaptability of seedlings to the transplantation site, for which the use of selected native beneficial microorganisms such as mycorrhizal fungi or plant growth promoting bacteria can be crucial. The targets of these programs can be diverse either focusing on the environmental restoration and climate change mitigation – e.g., carbon sequestration enhancement and/or ecosystem recovery after deforestation, fire, mining or fuel spill among other disturbances – , or with a more clear economic purpose such as the provision of wood and biomass, or the production of ectomycorrhizal edible mushrooms with high added value, e.g., truffles (Oliach et al., 2021), in agro-forest systems.

Given the increased frequency and duration of drought and natural disturbances, the raising costs of fertilizers and chemicals for nursery tree production, and the demand of consumers for new products such as edible fungi, the use of native bio-inoculants adapted to the plantation sites is emerging as a highly advantageous ecologic and economic tool for the forestry and agro-forestry industry.

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S2-O-01
"Circular agronomy" and Culturomics: Two sides of the same coin in the design of tailored low cost biofertilizers to promote plant growth under abiotic stresses

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According to the European Commission, global consumption of biomass, fossil fuels, metals and minerals is expected to double by 2050, while annual production of wastes will increase by 70%. In this context, the European Commission adopted the new circular economy action plan (CEAP) which intends to integrate development and sustainability, thus, the design of tailored biofertilizers is of the outmost importance.

The aim of this work is, using a culturomics approach [1], the isolation and characterization of specific PGPR and endophytes in order to generate a biofertilizer that promotes plant growth under different abiotic stresses and increase crops yield using fewer inputs.

In our project, *Mesembryanthemum crystallinum*, an autochthonous halophyte of the Andalusian marshes with medicinal properties, has been selected as model since this plant has been also proposed for phytoremediation of metals such as Cd [2] and Cr [3].

We have developed a low-cost culture medium based on *M. crystallinum* biomass, called Mesem-Agar (MA) to isolate bacteria from three compartments (rhizosphere soil, root endophytes and shoot endophytes) and compare them with isolates obtained on standard TSA medium. Two independent collections, one from each medium, were generated, all the bacteria were identified by 16S RNA gene sequencing, and their PGPR properties and plant tissue degrading activities determined, as well as the minimal inhibitory concentration for several toxic metals.

A higher number of bacteria were isolated on TSA than in MA (47 vs. 33), but interestingly, MA medium led to the isolation of specific *M. crystallinum* associated bacteria. Distinct patterns of PGP properties and cell wall degrading activities were found in both bacterial collections. Three microorganisms carrying a complimentary combination of PGP activities from each collection were selected in order to produce two different consortiums, whose effect on plant growth under abiotic stresses and phytoremediation capacity is being evaluated.

These results indicate the feasibility of a culturomics approach trough "low-cost media" based on plant biomass to isolate the most suitable bacteria capable of promoting plant health and growth under abiotic stresses. In this way, we have established a "circular agronomy model" in which bacteria help plant to grow and, in turn, a culture medium based on plant wastes support bacterial growth at low prices.

Keywords: Halophytes, PGPB, endophytes, culturomics, low-cost inoculants, circular economy, phytoremediation, heavy metals pollution.

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S2-O-02
Reverting Chlorosis in iron-starved tomato by PGPB involves a combination of bacterial siderophores and a systemic induction of Fe-uptake responsive genes

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Alkaline pH in soils reduces iron (Fe) availability limiting Fe-uptake and compromising plant growth. In plants, the main visual Fe deficiency symptom is leaf yellowing, chlorosis, due to a decrease in chlorophyll content. In dicots, plants activate Fe absorption mechanisms involving a H^+-pump to acidify the medium and solubilize Fe III, the reductase (FRO) than reduces chelated Fe III, and the membrane Fe II-carrier (IRT). Currently, only synthetic chelates are available for agriculture, so seeking for environmentally friendly alternatives is a must. *In vitro* production of bacterial siderophores has been reported but effective strains are not as frequent and the underlying molecular mechanisms by which siderophore producing-PGPB improve iron content in plants remain to be defined, as PGPB are able to improve plant fitness triggering multiple targets. In this context, the aim of this study was to i) select siderophore-producing from a large subset of isolated PGPB and ii) to evaluate the ability of two different siderophore-producing *Pseudomonas* sp. strains (Z8.8 and Z10.4) to revert chlorosis in Fe-starved tomato plants. From a total of 210 strains, 30% of them were selected for their *in vitro* ability to produce siderophores and identified by 16s rDNA sequencing. Among them, the most abundant group was conformed by *Pseudomonas sp.* from which Z8.8 and Z10.4 were selected by the size of the halo produced when growing in Cas-media with Fe, Mn or Co. The *Pseudomonas* Z8.8 was also able to produce auxins *in vitro*. After the inoculation of Z8.8 and Z10.4 in Fe-starving tomato plants, the chlorophyll content, the photosynthesis rates and the expression of Fe deficiency-responsive genes in roots (the plasma membrane H+-ATPase 1 *HA1*, the Fe(III) chelate reductase *FRO1,* and the iron Fe(II) root transporter, *IRT*) were assessed, as well as iron content. Chlorosis was reverted by Z8.8, with Fe increases around 40% as compared to controls, provided from $FeCl_3$ at alkaline pH. Photosynthesis performance was improved (ϕ PSII and Fv/Fm) as well as chlorophyll "a" content suggesting a systemic activation in plants treated with Z8.8. Opposite to our expectations, expression of Fe-uptake responsive genes was not enhanced on PGPB-treated plants at harvest time, rather the opposite. This evidences activation of innate plant mechanisms to obtain Fe in adverse conditions: the H^+ pump is highly active in the plasma membrane of iron starved plants in an alkaline environment to acidify soil and make Fe-III available to reductase. However, soil acidification is not needed when PGPB-siderophores are present, as bacterial Fe-chelates are going to make Fe more soluble and prone to be reduced by FRO in order to enter the root through IRT, immediately after bacterial-inoculation. It is consistent with the reported increase of Fe content, probably being an idirect mechanism apart from the direct chelant action of the bacterial-siderophore. However, gene expression has also been altered, being downregulated 9 days after bacterial inoculation, so we speculate that *FRO* and *IRT* genes have been systemically induced right after PGPB treatment improving iron nutrition provided by bacterial chelates. Therefore, although further studies are needed to confirm our hypothesis, Z 8.8 strain effectively reverts tomato chlorosis combining direct and indirect mechanisms of action.

S2-O-03
The combination of rice straw mulch with nitrogen fixers and plant growth-promoting bacteria (PGPB) has a synergic effect in crop production and soil microbiome

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Rice straw is an agricultural residue with high potential as mulch in woody crops where it exerts several benefits such as: preventing soil erosion, increasing water use, reducing weeds, increasing production, and/or regulating soil temperature (Prosdocimi *et al*., 2016; Ramakrishna *et al*., 2006). However, the rice straw decomposition process promotes the temporary immobilization of soil nitrogen due to its high C/N ratio (Williams *et al*., 1968). The incorporation of different diazotrophs into rice straw could compensate for this immobilization of nitrogen, as well as provide other benefits for crop production. In a microcosm assay, in trays 54 cm x 39 cm, we evaluated the effect of the combination of a rice straw mulch with the addition of different diazotrophs and other plant growth-promoting bacteria (PGPB), including one solubilizer of mineral insoluble phosphate and one siderophores producer. In the trays, the cultivated crop was ryegrass (*Lolium perenne).* The statistical design was a random complete block design with the treatments consisting of the combination of mulch with bacteria and the controls consisting of the corresponding uninoculated controls either with mulch or naked soil. The evaluated variables were, from the productive point of view, the aerial biomass produced by the crop, and its content in Nitrogen, Phosphorus and Iron; form the environmental point of view, the composition of the soil microbiome by next-generation sequencing of total soil DNA. Results of crop biomass production showed that, compared to naked soil, mulch produced decreased values during the cold months and increased during the warm months. Interestingly the inoculation with the selected bacteria strains improved the biomass production of the crop at any season, and some N-fixing strains even compensated the decrease in biomass production caused by mulch. Moreover, some N-fixers also compensated the reduction of bioavailable N caused by the increased microbial activity under mulch. Soil microbiome was positively affected by the mulch, because it increased biodiversity, both alpha and beta. In addition, inoculation hardly changed the structure and composition of the soil bacterial community; the main modifications were a slight increase of alpha biodiversity and a slight reduce of beta biodiversity. The inoculated bacteria did not persist in time and re-inoculation each season is necessary. These findings suggest that the incorporation of diazotrophs and PGPB into rice mulch would compensate for the disadvantages of mulching while synergic benefit to the crop.

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S2-O-04
A novel complex mineral fertilizer composition including rhizobia improves yield with a reduced dose

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It is well known that rhizobia exert a Plant Growth Promoting (PGP) action in non-legumes, resulting in an improved crop yield and quality (Flores-Felix et al. 2021). Moreover, it has been proved in field trials that a reduced dose of mineral fertilizers along with the inoculation of PGP Bacteria (PGPB) results in a yield improvement, compared to a full mineral fertilizer dose without inoculation (Pastor-Bueis et al. 2017). An European Patent registered an invention consisting on a granulated mineral fertilizer that includes rhizobia as PGPB to be used as a fertilizer for non-legume crops (Mulas et al. 2018). The novelty comprised the carrying system of the PGPB in the fertilizer granule and the PGPB dose that exerts an agronomic effect. Field trials at commercial scale demonstrated that the fertilization of cereals with the invention improved the crop yield compared with the fertilization with a plain complex mineral fertilizer. In concrete, in two field trials with wheat, the yield enhancement surpassed 5% compared with the control consisting on the mineral fertilizer without rhizobia. Moreover, the yield observed with the invention at a reduced dose (80%) of fertilizer surpassed not only the yield with the same dose of the plain mineral fertilizer, but also with the full dose (100 % that corresponds to the recommended dose) of the plain mineral fertilizer. The invention was further registered at the official Spanish registry of fertilizing products to be commercialized, and other field trials supported the results presented in this work.

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S2-P-01
Evaluation of *Gracilaria gracilis* effects on tomato plants: pipelining the use of a new alga extract as biostimulant

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Nowadays, considering the ever-increasing world population's demands, which is expected to reach 9.7 billion by 2050, food supply has become one of the most relevant problems. Conventional agricultural management models overused chemical pesticides and synthetic fertilizers inputs, causing a depletion in farms and ecosystems, and many pollution and health problems as well. Moreover, plant-related microbiota, which is being showed to be crucial in plant health and development, is very sensitive to this type management, generally leading to a biodiversity loss[1]. Consequently, we need to implement new sustainable alternatives in agricultural management in order to ensure the food production for the next decades.

Oceans have been providing a wide variety of extraordinary organisms with high relevance for agricultural management, such as seaweeds. In Portugal and many other seaside countries around the world, seaweeds were traditionally used in agricultural fields, providing organic matter, minerals, trace elements, growth-plant regulators, metabolites, vitamins and amino-acids [1,2]. More recently, several studies have been concluded that applying seaweed extracts to soil, positively affects the rhizosphere and phyllosphere microbiota patterns. For instance, *Ascophyllum nodosum* extract has been shown to improve plant growth, enhance beneficial microorganisms, mitigate some abiotic and biotic stresses, and improve plant defenses[1]. However, the use of these extracts as an alternative is facing the complexity to characterize them in composition, type of application, optimal plant stage, effective concentration, effects on microbiota…

In this context, our work project aimed to develop a pipeline to assess the effects of seaweed extracts treatments on *Solanum lycopersicum*. Potential effects of a novel seaweed extract made from the red macroalgae *Gracilaria gracilis* were evaluated at different concentrations, application way, and at different developmental stages. Results indicated that *G. gracilis* extract may enhance plant phenotype at 0.25% and 0.5% concentrations, showing a positive effect on seed germination rate and general plant development. Moreover, we decided to go further and characterize the effects on root/soil microbiota, as well as the possible connection with epigenetic marks through the evaluations of the methylation level of key developmental genes, as a complementary understanding of the plant-soil ecosystem effects of these extracts. Finally, we also detected the presence of some bacteria strains in the extracts so we included complementary characterization test of these strains as bioinoculant. Data obtained in this work may contribute to the better use of seaweed extracts in agriculture, although further characterization studies must be carried out to have a broader understanding on this.

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S2-P-02
Effect of bacterial inoculation on the physiological response of strawberry under phosphorus deficit

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The strawberry sector is a main economic and social driver in Andalusia (Spain), as a great generator of jobs and exportation profits. Despite its importance, the sustainability of its cultivation in the short and medium term is compromised. Intensive strawberry cultivation generates a great environmental impact due to the application of excessive chemical inputs that contaminate aquifers and soils. This situation is especially critical in sensitive areas, such as the Doñana National Park and its surroundings (SW Spain), where this crop may be found. Most of chemical fertilizers applied cope with the scarce availability of phosphorus in arable soils, an essential nutrient which is largely inorganic and insoluble for plants. Therefore, more environmentally sustainable alternatives must be designed to reduce the use of chemical fertilizers and still improve crop yields. Thus, the main objective of the present work was to evaluate the effect of a consortium of plant growth promoting rhizobacteria (PGPR) on the growth and physiological response of strawberry plants grown under phosphorus deficit conditions. The consortium employed included PGPR with the ability to solubilize phosphates, thereby increasing phosphorus bioavailability in the rhizosphere of the plants. For this study, four differential treatments were chosen depending on whether or not the plants had bacterial inoculum and whether or not the fertilizer used contained inorganic phosphorus. The results obtained showed, in general, a positive effect of PGPR inoculation, and this effect was especially noticeable in plants grown with an insoluble phosphorus supply. These plants showed a higher growth of above and below ground biomass, an improvement in processes related to photosynthetic efficiency and water use efficiency, as well as a higher content of photosynthetic pigments in the leaves. This response was mediated by direct and indirect effects of the bacterial strains used on the plants, especially the phosphate solubilization capacity, which made possible the bioavailability of the phosphorus provided, initially inaccessible to the plants. In short, this study shows the potential of PGPR inoculation to improve strawberry tolerance to phosphorus deficit, aiming at more sustainable cultivation practices in this crop.

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S2-P-03
Agronomic and environmental performance of rhizobial inoculants in common beans in the Dominican Republic

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The common bean (*Phaseolus vulgaris* L.), with more than 34 million ha and 30 million t worldwide, is an essential foodstuff for human beings (Broughton et al., 2003). The inoculants consisting of native-naturalised rhizobia selected because of their N fixation efficiency, so called elite autochthonous strains, are generally successful in increasing crop yield (Koskey et al., 2017). Adaptation to the local environment makes them highly competitive, which usually results in greater nodule occupancy, producing a good field performance, expressed in superior yield (Irisarri et al., 2019).The aim of this work was to assess the agronomic and environmental performance of the inoculants' technology in common bean crops in the Dominican Republic. For the environmental assessment, the Life Cycle Analysis (LCA) methodology was use. The LCA followed the ISO 14044 guidelines. These guidelines state that the first step is to define the goal and scope, the second is life cycle inventory (LCI), the third is the life cycle impact assessment (LCIA), and the fourth is life cycle interpretation. The results show that the use of the autochthonous elite strain and the formulation presented in this work increased the average yield by a 1.8% compared to the conventional technology. For the common bean, the highest environmental impact per ha corresponded to the conventional technology for all the impact categories. A shift to inoculation technology would reduce the environmental impact per ha in all the categories, ranging from 16% in fresh water aquatic eco-toxicity to 25% in acidification. In reference to one t of produced grains, the impact reduction would be slightly higher, by an additional 2%, on average, for each category. Interestingly, in reference to 1 ha, the inoculation technology barely increased the environmental impacts compared to the negative control that did not receive either N fertilisation or inoculation. When the environmental impacts were allocated to 1 t of produced grains, the inoculation reduced them by 21% on average, compared to the negative control, due to the yield increase due to the SNF. In addition, it is interesting to highlight that the conventional technology produces similar or even lower (1%-5% lower) environmental impacts than the negative control per t of produced grains; only the human toxicity was higher in the conventional technology than in the negative control. It is concluded that inoculation technology enables mineral N fertilisation to be replace, producing a weak yield increase (less than 2%) in common bean, when compared to the technology used today in the Dominican Republic. From an environmental viewpoint, the Greenhouse Warming Potential (GWP) and energy demand for manufacturing one unit of inoculant, define as the inoculant needed for 1 ha are less than 1% of those corresponding to the production of mineral N fertiliser. Consequently, this technological substitution reduces the environmental burdens per cropped ha and per produced t, for the categories of environmental impact analysed, although in different percentages. For common bean, the reduction ranged from 16% to 25%, and was similar per ha and per t.

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S2-P-04
And what is going on with MPB in real field situations?

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As a consequence of the laying down of the EU 2019/1009, the use and the licensing of Microbial Plant Biostimulants (MPB) in agriculture have received a boost (Pastor-Bueis et al. 2019). According to the European regulation, the MPB that can be licensed are strains belonging to the genera *Rhizobium, Azotobacter, Azospirillum* plus mycorrhizal fungi (Europe, 2019), but the regulation opens the door to increase the number of accepted genera including other Plant Growth Promoting Microorganisms (PGPM). The expected role of MPB according to EU2019/1009 is not only N-fixing both in symbiosis rhizobia-legumes and free-living *Azotobacter* and *Azospirillum*, but also the abiotic stress alleviation, the growth stimulation and the quality improvement, thus resulting in better yield in quantity and quality. The academic research about PGPM is very relevant, and much information is publicly available referring to the metabolism of microorganisms beneficial for agriculture, using cutting edge omics technology. Such information is very important for “product developers”, because help them to select strains based on specific functional actions, which is more efficient than the selection based on screening tests of hundreds of isolates to find "by chance" an expected action in the crop. However, the agronomic tests of selected strains or of fully formulated MPB are less available in scientific literature (Barquero et al, 2019), because most of the agronomic tests are carried out as part of the requirements for registration at the national regulation bodies and subsequent licensing. In this work, we present a compilation of field trials at medium and large scale that have been published in the academic literature, and that demonstrate the effectiveness of MPB to improve the yield in a scenario in which the nutrients are provided in a reduced rate. The analyzed works gather different kind of MPB, including: i) specific rhizobia selected for legume crops in which inoculation fully replaces the N mineral fertilization; ii) other PGPB for non-legume crops with reduced doses of mineral fertilisers. In general terms, the MPB improve crops yield compared with the control that receives fertilization in a conventional way. The inoculation of legumes with specific autochthonous rhizobia in absence of a source of mineral N in all the field trials produced at least the same yield than the fertilization with mineral N without inoculation, but interestingly in some trials the inoculation produced even more yield (up to 28% more) than the fertilization. In the case of non-legume crops inoculated with PGPB at a reduced dose of mineral fertilizer, the yield increase compared to the uninoculated controls fertilized with a full mineral dose was up to 34%. However, the good results obtained with the use of MPB hide a threat, because it has been demonstrated a high dependence of the inoculated crops from the high level of nutrients existing in the soil (a legacy owing to applications of fertilizer during many years) (Pastor-Bueis et al., 2019). Indeed, undoubtedly MPBs enhance Nutrients Use Efficiency and thus a question arises: if the fertilizers dose is reduced and the MPBs enhances the use of the nutrients reservoir in the soil, what is going to happen when such a reservoir diminish under an unknown limit?

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S2-P-05
Physiological and genetic modifications in tomato plants subjected to moderate water stress and elicited with two plant growth promoting bacteria (PGPB)

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In Spain approximately 80% of fresh water is used in agriculture and is essential to maintain agricultural production. It is a very high percentage in a country where water has always been a scarce commodity. If we add to this that climate change is progressively aggravating this problem, reducing the amount of water used in agriculture must be a priority challenge. Until now, the problem has been addressed almost exclusively by trying to improve irrigation systems, but little investment has been made in trying to keep plants productive in situations of moderate water stress. The objective of this work is to study the physiological and genetic changes produced by two strains of the Pseudomonas sp. (N 5.12 and N 21.24) inoculated in tomato plants subjected to moderate water stress, which do not cause wilting, and which can ensure that the plants maintain their productivity, thus reducing the amount of water, which would result in a decrease in water used in agriculture. For this, tomato plants one month from sowing were inoculated with the strains twice, one week apart. Three days after the second inoculation, the plants were subjected to water stress using 10% polyethylene glycol 6000 (PEG6000). Throughout the experiment, the plants were watered with 20 ml of water every 2 days. To cause moderate water stress, the plants were irrigated with 20 ml of PEG6000 at 10%, except for the control plants that were irrigated with water. Three days after water stress, the photosynthetic capacity of the plants was measured, and the plants were collected to perform the different analyses. The oxidative stress of the plants was measured by measuring the concentration of H_2O_2, concentration of malondialdehyde (MDA) and the activity of the enzymes ascorbate peroxidase (APX) and Glutathione reductase (GR). The concentrations of proline, glycine-betaine and soluble sugars as main compatible solutes were measured and the differential expressions of P5CS (Pyrroline-5-carboxylate synthase, related with proline biosynthesis), NCED1 (9-cis-epoxycarotenoid dioxygenase, related with ABA biosynthesis) and PM ATPase 1 (plasma membrane ATPase 1) genes were studied. Both strains are able to maintain net photosynthesis at control levels and significantly higher than plants treated with PEG alone. Furthermore, plants treated with N 21.24 had transpiration rates like those of the control, while plants treated with N 5.12 had transpiration rates significantly lower than the control and like plants treated with PEG. N 21.24 causes an increase in glycine-betaine but not proline or soluble sugars. Unlike N 5.12 which increases proline but not the other compatible solutes. The two strains are able to decrease the concentration of H_2O_2 in the plants with respect to the plants treated with PEG despite the fact that the APX and GR activities were lower. Of the genes studied, the significantly higher differential expression in the NCED1 and ATPase genes in the plants treated with N 21.24 is very striking. The higher expression of NCED1 does not result in stomatal closure, but it is reflected in the decrease in the concentration of carotenoids and in an increase in the concentration of glycine-betaine (Nawaz et al., 2020), probably the greater expression of ATPase 1 involved in the opening of stomata is causing the stomata not to close and high transpiration rates are maintained. The results obtained indicate that the strains used, especially N 21.24, cause physiological and genetic changes compatible with plants better adapted to conditions of moderate water stress, so it could be used as a biotechnological tool in real farming situations, in which it could be reduce the amount of irrigation without affecting the performance of the plants, obtaining real water savings.

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S2-P-06
Bacillus H47 activates DOXP and shikimate pathways modifying olive leaf antihypertensive activity under water stress

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Despite the innate adaptative mechanisms of plants to survive to different environmental stresses, these can further be improved by other means such as beneficial bacteria. Effective PGPB strains are able to trigger multiple targets in the plant resulting in a tailored adjustment to adverse condition. Stimulation of secondary metabolism is of special interest as certain secondary metabolites are able to bind human receptors improving human health; so if metabolites accumulate in edible parts, health benefits are achieved through the diet, while accumulation in non-edible parts may be transformed in economic profit by transforming agricultural debris into extracts with bioactivity, contributing to circular economy. The secondary metabolism of Olea europaea includes polyphenols with a general antioxidant activity as well as specific antihypertensive molecules (iridoids) among others. In this work, the capacity of three Bacillus strains to stimulate the secondary metabolism of one-year-old olive seedlings subjected to salinity stress was studied along 12 months, during which root inoculations of the three strains were performed. In October (month 12) photosynthesis, photosynthetic pigments, bioactives (iridoids and flavonols) and a gene expression study of the main enzymes of the synthesis pathway of these secondary metabolites was performed.

The three strains were able to improve the energy machinery of the plant, improving CO2 fixation, increasing energy dissipation in the form of heat, reducing oxidative stress. *Bacillus* H47 was found to trigger synthases in the DOXP pathway (up to 5-fold in DOXP-synthase, 3,5-fold in Iridoid synthase and 2-fold in secologanin synthase) associated to a concomitant increase in iridoids (up to 5-fold in oleuropein and 2-fold in its precursor secologanin). Regarding the oleuropein precursor, hydroxytyrosol did not accumulate in the leaf according to HPLC analysis, nor did it increase the expression of its key synthesis enzymes. This suggests that it could be due to a mechanism to block the natural feedback inhibition of the pathway, since oleuropein is increased about 5 times compared to control plants. On the other hand, the increase in flavonols (rutoside and lutelin-glucoside), is not associated to enhanced gene expression of its key synthesis enzymes, following the pulse activation model proposed for innate immunity. Finally, the ACEI activity (inhibition of Angiotensin Converting Enzyme) was not improved, suggesting translocation and accumulation of antihypertensive metabolites to the fruit, and consequently to the oil. In summary, Bacillus H47 is a PGPB strain capable of improving plant adaptation to salinity stress or water scarcity, activating photosynthesis and secondary metabolism.

S2-P-07
Maize crops under rising temperatures: the effects of plant growth promotion rhizobacteria

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Different models of climate change generally describe an increase in planet surface temperature. This increase will impact species and ecosystem function and will affect agricultural production. Added to these extreme temperatures, world population is expected to increase, imposing a pressure on food production. Nowadays there is an effort to produce food in a sustainable way, being plant growth promotion bacteria one of the most promising methodologies. Thus, it's essential to understand how the plant-bacteria interactions will change in high temperature scenarios in order to better delineate crop production strategies.

In this study, maize plants (*Zea mays*) were exposed to 26 °C and 35 °C for one month. Three conditions were tested: non-inoculated plants; plants inoculated with *Pantoea dispersa*; and plants inoculated with *Herbaspirillum huttiense*. These strains were chosen for evidencing several plant growth promoting traits (IAA and siderophores production, phosphate solubilization, antifungal capacity and volatile promotion) at elevated temperature. Results were evaluated by morphometric, physiological and biochemical parameters. Lipidomics and metabolomics were also carried with the bacterial strain displaying the best results - *Pantoea dispersa.*

Use of a multivariate analysis evidenced a clear separation among conditions at each temperature. At both temperatures bacteria were able to increase the leaves and root weight, the response of the enzymatic antioxidant system, protein, sugars and chlorophyll B. Additionally at 35 °C the metabolic activity, starch and proline also increased and there was a decrease on membrane damage compared to control (non-inoculated plants) at the same temperature. Both temperature and bacteria influenced the lipidomics and metabolomics profiles.

Results show that temperature tolerant PGPR can play an important role on the protection of plants, such as maize, to high temperature in greenhouse conditions. These results should first be confirmed on field trials before they can be considered as a strategy to increase the resilience of crops to global warming.

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S2-P-08
Development of a biofertilizer based on biochar and PGPR bacteria in combination with compost

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Global pollution is one of the main environmental problems we face today, and an excessive use of chemical in agriculture causes harmful effects on the environment that increase this problem. It is well known that in order to achieve an adequate crops yield, the agricultural soils must be supplemented with nutrients in the form of mineral fertilizers (Kandpal, 2021). However, products based on PGPR (Plant Growth Promoting Rhizobacteria) might contribute to reduce the use of mineral fertilizers obtaining similar or even higher crops yield (Bhattacharyya & Jha, 2011). By the other hand, biochar has become popular in agriculture because it improves physical, chemical and biological soil properties and moreover it has been described as a carrier for PGPR (Pastor-Bueis et al., 2019). This work attempts to study different formulations of biofertilizers as a partial substitute for conventional mineral products. Such biofertilizers consist of PGPR bacteria and biochar as a carrier, in combination with compost.

The assay was carried out in microcosms conditions in a greenhouse at the University of León (Spain) with the purpose of evaluate the agronomical effects in ray-grass, of different formulations of biofertilizers. A total of 14 treatments including the corresponding controls were evaluated. The treatments differed in the rates of the different components i.e. the compost and the PGPR formulated in biochar as carrier. The biochar was produced from olive tree pruning with a pilot reactor in continuous mode, at a working temperature of 600°C for 40 min of solid retention time. The PGPR was *Bacillus* strain. The statistical design was completely randomized, with six repetitions per treatment. The parameters analysed were the fresh and dry biomass produced by the rye-grass, and the results were statistically analysed with ANOVA.

Results of fresh and dry biomass produced by rye-grass, showed significant differences between the non-fertilized control and the treatments with the biofertilizer. Moreover, the best treatment was the combination of compost, and the microorganism formulated with biochar, proving the viability of using this organic fertilizer because of its agronomic potential and its contribution to reduce the use of chemical products. Besides, the developed product aligns with the principles of circular economy, reusing olive pruning as a biochar, that causes great environmental issues in regions with vast areas with olive tree crops.

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S2-P-09
Effects of PGPB inoculation on the soil microbiome

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Increasing crop productivity while minimizing environmental damage is one of the main agricultural priorities nowadays. Plant growth-promoting bacteria (PGPB) are considered an ecological alternative to improve crop productivity. Then, the inoculation of PGPB to crops is increasingly used but its potential effects on soil biodiversity are largely unknown. These effects are often forgotten when new agricultural practices are proposed to improve crop productivity. Soil health could be both impacted positively and negatively as a result of PGPB inoculation. Biodiversity is key to a balanced soil ecosystem and, interestingly, can enhance soil resilience against environmental disturbances. The assessment of PGPB-induced effects on not only crop plants but also soil functioning is fundamental for the safe use of bioinoculants as biofertilizers. The specific bacterium used in this study (*Micromonospora cremea)* is a soil inhabitant (Carro *et al*., 2012), but its application in high amounts can induce structural changes in soil microbial communities. Hence, it is essential to properly identify and understand such changes, as well as to evaluate soil resilience to the inoculation-induced effects, in order to warrant the eco-sustainability of our bioinoculant in terms of its effects on soil agricultural health.

To this purpose, after inoculation of *M. cremea* in pea plants, we monitored the changes in soil microbial diversity and, concomitantly, soil resilience. Using a variety of soil properties as endpoints, we monitored soil, plant, and microbial responses to PGPB inoculation over time (0, 7, 28, and 98 days after inoculation) in the absence *vs.* the presence of water stress, in order to partially mimic the current scenario of climate change. Our results show a direct impact of *M. cremea* inoculation on soil properties, and specifically on soil microbial diversity. This impact was then partially buffered by the natural soil resilience. More studies are needed to study the potential effects *M. cremea* multi-applications and yearly additions, in order to keep on assessing its safety as a PGPB bioinoculant.

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S2-P-10
Encapsulation of rhizobacteria and bacterial volatiles as biostimulants: application in the promotion of plant growth

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Maize (*Zea mays*) is one of the most important food crops in the world, since along with rice and wheat, it provides 30% of food calories to over 4.5 billion people in 94 countries (Shiferaw et al. 2020; Abbade 2021). We are currently dependent on agricultural profitability and facing vulnerability due to climate change to feed an increasing human population. Thus, new approaches are necessary to meet the production needs in a sustainable way.

Some soil bacteria produce volatile organic compounds that can promote plant growth. The use of these bacteria or the volatiles they produce can be an alternative and efficient way to promote plant growth and tolerance to abiotic factors.

In this study it is proposed to use bacterial volatiles, already identified to promote plant growth (Zou et al. 2010), and inducers of maize growth and tolerance to drought, and to encapsulate these volatiles in alginate. The results evidenced the positive effect of encapsulated volatiles in the growth of water stressed and non-stressed maize plants. The encapsulated volatiles 2,3-butanediol and 2-pentylfuran at a concentration of 18 µg showed positive growth relative to control. These results are promising and can be a starting point to launch new solutions to grow crops less dependent on traditional inorganic fertilizers, using water more efficiently and promoting a more sustainable agriculture. The aim is to reduce the consumption of water and chemical fertilizers in agricultural fields, while improving agricultural productivity.

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S2-P-11
Improvement of agronomic performance and fruit quality parameters with an organic fertilizer amended with *Bacillus* sp.

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The use of renewable biomass resources (bio-residues) as a complement to agricultural fertilizers has been promoted by the European Union through the Circular Economy. These bio-residues must be conditioned for use as agricultural inputs (De Corato, 2020) before being applied to crops. The introduction of circular economy principles in agriculture (Cong and Thomsen, 2021) combined with fertilizer-based techniques with others based on microbiology (Backer et al. 2018) represents an interesting and promising solution to solve the problem of excess chemical inputs. The bio-residues treated by composting and pyrolysis processes and used in this research were compost and biochar, well known for their applications in agriculture. The aim of this study was to evaluate the agronomic and quality-related effects on melon fruit after the application of an organic fertilizer. This fertilizer consisted of an amended compost with a bacterial strain of the *Bacillus* genus with plant-growth promoting (PGP) properties and biochar as a carrier.

A field trial was conducted on melon crop using a randomized complete block design with three blocks in Murcia (Spain). The soil treatments applied before transplant were compost 5 t/ha with 6% amendment, compost 5 t/ha without amendment, a control with mineral fertilizer and a blank. The dependent variables of the crop measured were: yield, number of fruits per hectare, dry aerial plant biomass; and the fruit quality-related dependent variables were: fruit weight, fruit contour, penetrometry, conductivity and solute concentration.

The best results were obtained for the amended compost treatment when compared to the mineral fertilizer for both agronomic and fruit quality parameters (except penetrometry parameter) with improvement percentages resulted in 14% and 97% increase depending on the parameter measured. These results indicate the huge agronomic potential of organic fertilizers combined with microbial stimulants in this formulation to contribute to the partial reduction of mineral input levels and the promotion of sustainable agriculture.

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S2-P-12
Alfalfa-*Sinorizobium* rhizospheric communication in adverse thermal conditions

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Root hairs exudate compounds that act as a chemotactic signal or promote the growth of symbiotic fungi and bacteria. Moreover, they are directly involved in the formation of nitrogen-fixing nodules in legumes, secretes signaling flavonoid compounds that are perceived by the rhizobial symbiont, which responds to this message by secreting specific lipochitooligosaccharides, named Nod factors (NF). NF are signal molecules whose binding to root hair receptors triggers complex signaling events leading the root hair to curl and thereby to entrap rhizobia. Then, an infection thread develops, allowing rhizobia to migrate through the root cortex toward the nodule primordium. In alfalfa, they elicit root-hair deformation, cortical cell divisions and the formation of genuine nodules. The synthesis and secretion of flavonoids and NF by different legume–rhizobia interactions were found to be altered under environmental stress conditions (Guasch-Vidal et al., 2013). Among environmental factors, temperature is the one exerting the strongest impact on the cell envelope of *S. meliloti*, modifying its lipid composition and its biophysical state, which can affect the establishment of the symbiosis with alfalfa (Paulucci, et al., 2021). Although the effect of NF secreted by *S. meliloti* under optimal conditions on early interaction events with alfalfa roots is known (Damiani et al., 2016), there is a lack of information on what could be caused by those NF secreted under non-optimal conditions, such as temperature. For this reason, the objective of this work was to evaluate the effect of the FN produced by *S. meliloti* induced with luteolin at different stages of the thermal cycle (28°C-10°$C_{Initial}$-.40°C-10°C_{Final}) on the curvature of alfalfa root hairs.

We observed a higher number of root hair curvature events (distortion, shepherd's crook and tip bending) in alfalfa roots inoculated with FN obtained from cultures of *S. meliloti* induced with luteolin after exposure to 40°C. This result is in agreement with that obtained in alfalfa inoculation tests with *S. meliloti* previously exposed to 40°C. There we were able to observe a greater number of average nodules per plant (40±9) compared to the other conditions (24±9 and for 23±2 for 28°C and 10°CInitial respectively and 18±6 for 10°CFinal). In addition, when the roots were inoculated with FN secreted by *S. meliloti* in the 10°CFinal condition, root hair curvature structures different from the typical ones were observed.

These results suggest that the FN produced by *S. meliloti* in the heating stage of the applied thermal cycle could improve the early molecular dialogue with alfalfa and the establishment of the symbiosis. Future studies of our working group are focused on elucidating the composition of the FN in this thermal condition.

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S2-P-13
Innovative inoculants: The use of legume seeds with optimized microbiomes

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The use of microbial inoculants in agriculture is one of the key technologies to ensure the biodiversity, sustainability and productivity of this sector. In the case of nitrogen fixing bacteria (rhizobia), the selection for the optimal combination of these bacteria and the host legume usually results in more effective symbioses and better growth of the host legume plant. The inoculation of legumes with nitrogen fixing bacteria, such as legumes for permanent pastures, is a widely used practice. Nowadays, innovative legume inoculants should also contain nitrogen fixing bacteria selected to other functionalities in the face of abiotic and biotic (phytopathogenic agents) stress factors with greater negative impact in agro-silvo-pastoral systems. However, the formulation of inoculants with reliable and consistent effects is still a constraint for their use. To overcome this, the use of liquid inoculants amended with substances that could improve stickiness and adherence to seeds and also the viability of bacteria in the seeds is an important issue.

The aim of this work was to study autochthonous nitrogen fixing bacteria strains isolated from several *Trifolium* spp. grown in the South of Portugal and to select them according to their symbiotic performance. For this purpose, experiments in controlled environment conditions were performed to evaluate the symbiotic effectiveness of different species of *Trifolium* spp. inoculated with several rhizobia strains. *In vitro* assays were also performed to evaluate the antagonist activity of the pre-selected bacterial strains against *Phytopythium* sp. and *Phytophthora* sp.. These oomycetes have been associated with decline of agro-silvo-pastoral ecosystems. Two bacterial consortia containing 4 and 5 pre-selected strains, respectively, were used to inoculate different species of annual clovers and their symbiotic performance in controlled conditions was evaluated. It was also tested the inclusion in the consortia of a *Lysobacter* sp. strain (Soares et al. 2021) (previously isolated from the root nodules of *Lotus* spp.) which was shown to have great antagonistic activity against the oomycetes tested. Additionally, one type of additive to use for seed inoculation was also tested, as an alternative to the traditional formulation of inoculants, namely a seed coating agent that is commonly used as adhesive in legume seeds inoculation. The tests were performed using also seeds inoculated without any additive, as controls. These assays were done to evaluate the seed bacterial viability over time. The results showed that most of the strains tested were symbiotically effective with the different annual clovers. However, most of them were not antagonistic to the 2 oomycetes tested. Nevertheless, the results obtained in the essays carried out with different annual clovers inoculated with the 2 selected microbial consortia, showed the best symbiotic performance when in the presence of *Lysobacte*r. In the viability tests of the bacteria inoculated in the seeds of *Trifolium subterraneum*, although there was a decrease in the number of bacteria per seed, in both treatments tested, the number was still high 5 weeks after inoculation. These results could allow the implementation of pastures or other soil covering crops, more profitable and sustainable in the long term, bringing real benefits to pastures including health control.

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S2-P-14
Selection of plant growth promoting bacteria for inoculation of pasture grasses

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The inoculation of plants with beneficial microorganisms has become a common practice in agriculture, and it is nowadays accepted that plant growth-promoting bacteria may represent a sustainable way to improve crop production with less dependence on synthetic products. In addition to the well-known ability of legume plants to associate symbiotically with nitrogen-fixing rhizobia, non-legume plants also establish associations with beneficial bacteria, and the development of inoculants for these crops may represent a plausible way of supplying a significant part of their requirements. However, there is still a lack of knowledge regarding inoculants for many non-legume crops, with few and little specific products on the market. This is the case of pasture grasses, such as annual ryegrass (*Lolium multiflorum* Lam.), a fast-growing, cool season pasture and forage crop. Annual ryegrass is commonly included in biodiverse mixtures of legumes and grasses that are often sown in agro-silvo-pastoral systems ("montado" or "dehesa") in the southern Iberian Peninsula, for animal grazing and forage. Here the soils are generally poor and affected by severe constraints, such as drought, acidity (with associated metal toxicities), low carbon concentration, low availability of nutrients, and easy spread of soil-borne diseases. In these systems, the inoculation of pasture grasses with plant growth promoting bacteria well adapted to extreme conditions could contribute to more productive and resilient ecosystems.

Previous surveys of annual ryegrass-associated bacteria in "montado" soils led to the isolation of several strains, some of them with plant growth promoting traits (Castanheira et al. 2014 and unpublished work). Here we describe the characterization of a set of such bacteria, with a view to establishing consortia for inoculation of annual ryegrass and other pasture grasses. The bacteria were identified by 16S rRNA gene sequencing and evaluated for plant growth promoting activities, as well as for the ability to grow at supra-optimal temperatures (35 ºC, 40 ºC, 45 ºC), low pH (4.5), and high Mn concentration (1-2 mM). Inoculation assays of *L. multiflorum* in synthetic medium assessed the stimulatory effects on plants biomass. These evaluations resulted in the selection of five bacteria (three *Pseudomonas* sp., one *Rhizobium* sp. and one *Paenibacillus* sp.) to integrate the inoculation consortia, covering a range of plant growth promoting activities (indole-3-acetic acid production, phosphate solubilization, siderophore production, cellulose hydrolysis, manganese oxidation) and ability to tolerate adverse conditions. Two strains showed antagonistic activities to the oomycetes *Phytophthora cinnamomi* and *Phytopythium vexans*, which have been associated with the decline of "montado" and pre-emergence damping off. The selected bacteria were tested for possible antagonisms between them. Assays are underway to assess the effects of additives on the shelf life and viability of the inoculants, as well as the effects of different combinations of the bacteria (consortia) in inoculated plants.

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XVIII SEFIN OEIRAS '22

BeMiPlant

Beneficial Plant-Microbe Interactions

SESSION 3

Beneficial Microbes for Soil and Environment

S3-L-01
Social PGPMs for future biofertilizers

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The industry of microbial bioproducts with a desired function is increasing in different sectors and is key in the Agro industry for biofertilizers. However, the study of microorganisms with PGPM effects and their posterior application is tedious, and these tend to contain single or dual microbial bacterial species in their formulation. Previous work in the field of biodegradation, launched the BSocial webtool to decipher the social behaviour of individual species. The results of mixing social bacterial species (with positive and neutral social behaviours) resulted in an increase in functional outcome, as well as corroborated the stability-diversity hypothesis which predicts more stable communities as diversity increases (Purswani et al 2017, 2019). Both the stability-diversity hypothesis and higher functional outcomes have also been corroborated between bacterial and fungal species (Angeles de Paz, et al 2022). Hence, the use of social microorganisms applied to biofertilizers, is key in increasing its stability when delivered to the highly diverse soil, while possibly increasing PGPM function too. Nevertheless, microbial soil studies are needed to observe natural PGPM and their social interactions to determine which microbial species may be best applied together under global warming conditions. To find social PGPM in soil capable of withstanding global warming changes, we exerted combinatorial temperature (4°C, 20°C, 50°C) and humidity (dry - 5%, field capacity – 25%, saturation - 50%) pulse stresses on soil microcosms, and determined microbial diversity (via 16S rDNA Illumina sequencing) at different time points and function recovery (nitrification activity). Time series dataset of the microbial diversity was used to decipher microbial interactions via the MetaMIS tool. During the study, we encountered: total functional recovery was observed within the time tested for stressT50H25 (temperature 50°C and humidity at field capacity). Constant recovery rates were found for stresses T20H5 and T4H5, i.e. drought conditions but mild temperatures. Nevertheless, nitrification activity was not recovered nor appeared to be recovering after drought and high temperature stress (T50H5). High temperature stress (T50), was the factor that increased microbial diversity dissimilarity distance between control samples (T20H25), followed by an increase in humidity, i.e.T50H5 did not cause the most dissimilar diversity. The network analysis of the social interactions of the time series control and T50 stress samples, together with the reference PlaBase database, describe a consensus network where social interactions of PGPM do not only occur among PGPM, but seem to be enhanced by microorganisms such as *Luteolibacter* and *Candidatus Nitrososphaera.* Hence, the use of social microbial network which include social microorganisms from the genus *Luteolibacter* and *Candidatus Nitrososphaera* should be tested in the future to enhance social interactions in biofertilizer composition.

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S3-O-01
Type VI Secretion System: a bacterial killing machine and biocontrol weapon

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The T6SS is a bacterial nanomachine used to inject effectors (toxins) into target cells. The system is found exclusively in Gram negative bacteria and frequently targets other prokaryotic cells; thus being considered a potent antimicrobial weapon.
Our model organism, *P. putida*, encodes three T6SS systems (K1, K2 and K3). Each one of those contains the complete set of genes to encode the core components necessary to assemble a functional machinery (Tss components), accessory components (Tag proteins) and over eleven effectors including nucleases and pore-forming colicins together with their cognate immunity pairs (Tke1-Tki1, Tke2-Tki2, Tke3-Tki3, …). Among these systems, the K1-T6SS is a potent antibacterial device considered a biocontrol mechanism used by *P. putida* to kill a broad range of bacteria, including resilient phytopathogens such as *Agrobacterium tumefaciens, Pseudomonas syringae, Pectobacterium caratovorum* and *Xanthomonas campestris*[1].
The structure of the T6SS resembles an inverted bacteriophage with a tube (Hcp proteins) surrounded by a contractile sheath (TssBC proteins) and capped with a puncturing tip (VgrG trimer). The cytosolic part of the T6SS docks onto a membrane complex (TssJLM) by interacting with a phage baseplate-like structure (TssAEFGK). Upon contraction of the sheath, the tube-tip complex, loaded with the effectors, is ejected and penetrates the target cell. The system is then disassembled and partially recycled for the next round of firing by the ATPase ClpV.
Although the structure of the T6SS is very well conserved, some systems contain variations of core components (TssA) that can be coupled to accessory proteins (TagB) allowing to fine-tune different mechanisms including the assembly and/or the firing of the system. We have described a novel mechanism for the T6SS sheath stabilization with implications in the system dynamics[2]. We are currently working on the system assembling and the mechanism of recycling as well as the regulation of the three systems to enhance phytopathogen killing. Importantly, we are testing the capability of *P. putida* to kill the quarantine pathogen *Xylella fastidiosa* -a threat for olive trees and other important crops of our country- using the T6SS.

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S3-O-02
Exploring soil Plant Growth Promoting Rhizobacteria potential to control Plant-Parasitic Nematodes: the case of *Phyllobacterium* and *Paenibacillus* against the pinewood nematode *Bursaphelenchus xylophilus*

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Plant-parasitic nematodes (PPNs) are considered major threats for agriculture crops, causing huge economic losses worldwide. Although the application of chemical nematicides is the most common method to reduce PPNs populations, their application has harmful effects on the environment and human health (Mingunola & Sasanelli, 2021). In this sense, the use of microbial nematicides (fungi and bacteria) represent a more environmentally friendly approach to partially or fully mitigate the damage caused by PPNs (Gamalero & Glick, 2020; Vicente et al., 2022). Plant growth promoting rhizobacteria (PGPR) have been raising interest as biocontrol agents against PPNs. The production of hydrolytic enzymes, toxins and volatile compounds are among the described mechanisms that bacteria may use to fight PPNs; however, the mode of action of PGPR against PPN and their interactions are not yet fully understood. In this work, we screened a collection of 40 isolates obtained from different cropping systems, displaying PGPR traits and biocontrol potential of fungal pathogens against two of the most devastating PPNs: *Bursaphelenchus xylophilus,* the so-called pinewood nematode (PWN) and causal agent of Pine Wilt Disease, and the root-knot nematode (RKN) *Meloidogyne javanica,* known to infect a wide range of agroeconomic valuable crops. *In vitro* tests of bacteria (pure culture, filtrates, and lysates) *versus* PWN/RKN revealed that many of the tested strains have nematostatic or nematicidal properties. The most effective bacterial strains belonged to the genera *Paenibacillus*, *Pantoea*, *Staphylococcus*, *Phyllobacterium* and *Sinorhizobium. In planta* tests involving PWNs and a susceptible host pine, *Pinus pinaster,* showed that the strains belonging to *Phyllobacterium*, *Paenibacillus* and *Staphylococcus* have the best results promoting the growth of *P. pinaster*. Remarkably, the estimated number of living nematodes within pine tissues was lower in the case of *Phyllobacterium* and *Paenibacillus* strains. The *in silico* study of their genome sequences confirmed the potential PGP activities, presence of hydrolytic enzymes and other genes that might be involved in the strains' nematicidal activities against several PPNs. Although further research is needed, our results revealed the potential of these PGPR strains as biocontrol agents against phytoparasitic nematodes, opening the possibility for the commercial formulation of a nematicide product.

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S3-O-03
Antibiosis or symbiosis? Characterizing the *Sinorhizobium fredii* USDA257 type VI secretion system

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Rhizobia are beneficial microorganisms to their plant hosts, the agriculturally important legume plants. Rhizobia and legumes develop a symbiotic relationship where the bacteria fix atmospheric nitrogen that the plant uses to grow and the plant supplies nutrients for the bacteria to live. The fixation of nitrogen by the rhizobia takes place in a newly developed organ located in the plant roots and induced by the rhizobia, the nodules. This process decreases the demand for chemical fertilizer that legumes would require for optimal growth. Thus, rhizobia are considered green fertilizers and their use contribute to a more sustainable agriculture. Our model organism, *Sinorhizobium fredii* USDA257, is one of the best characterized rhizobia. It is a fast-growing rhizobium with the capacity to nodulate a great variety of legume plants including *Glicine max, Vigna unguiculata* and *Lotus burtii*[1]. Among the molecular mechanisms best known for the symbiotic process and the determination of rhizobial host-range, is the Type III secretion system (T3SS)[2]. In recent decades, a novel gram-negative bacteria secretion system was discovered and named Type VI secretion system (T6SS). T6SS secretes effectors into prokaryotic and eukaryotic cells but has been mostly involved in interbacterial competition[3]. Curiously, T6SS is widespread in rhizobia, raising the question of whether it could also be involved in symbiosis as T3SS is. Here, we aim to elucidate the main role of T6SS in *S. fredii* USDA257 to answer the open question: antibiosis or symbiosis? For that, we have identified, by an *in silico* study, a solo T6SS cluster that contains all the genes necessary to encode the core components of the main structure (TssABCDEFGHIJKM), four genes encoding regulatory components (TagF, Fha, PpkA, PppA), three genes encoding accessory proteins (TagLMY) and two genes encoding hypothetical effectors (Tre1 and Tre2) that could be targeting eukaryotic cells. We have used the secretion of the inner tube protein, Hcp, as a hallmark of T6SS functionality to show that USDA257 T6SS is active and further induced in minimal media *e.g.* YM3 and MM. Competition assays between USDA257 and different preys (*Escherichia coli* and several phytopathogens such as *Agrobacterium tumefaciens*) showed that USDA257 cannot kill them using its T6SS in the tested conditions. Strikingly, nodulation assays with USDA257 natural host, *Glycine max* var. Pekin showed that plants inoculated with a USDA257 mutant with an inactive T6SS contained fewer nodules than plants inoculated with the USDA257 parental strain. The same trend is observed for the fresh mass of the nodule and the dry mass of the plant top parameters. These three parameters are indirect indicators of the capacity of rhizobium to develop the symbiotic relationship with the plant. Thus, the fact that the T6SS mutant had lower levels than the wild-type strain for all indicators could imply that T6SS might play a positive role in rhizobia-legume symbiosis.

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S3-P-01
Underestimated role of nodulated roots for soil fertility and quality

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Nitrogen (N) storage in legumes is usually estimated by N_2 fixation in shoots, whereas there is little knowledge on the contribution of roots and nodules to legume N and soil N. Here, we studied the contribution of recovered roots and nodules of grain and pasture legumes to plant N and soil N in Mediterranean fields. Experiments were run under rainfed conditions for a 2-yr period in three regions of Portugal (central and south west, and south inland). Complete plants including top plant and visible roots and nodules were sampled at the end of the growing seasons for grain legumes, sweet and yellow lupines, and over two harvests in case of pastures. N_2 fixation was measured by the ^{15}N techniques. Results showed that aboveground N concentration did not vary significantly among species, but differed in the belowground tissues (Carranca et al., 2015). Roots and nodules contributed to 7 – 11% of total legume N, with an allocation of 11 – 14 kg N fixed t^{-1} belowground dry weight (DW) in indeterminate legumes, representing half amount of aboveground plant. This finding demonstrated that investigation relying only on shoot N underestimates the role of legumes for soil N fertility. Further long - term studies on the contribution of belowground tissues to the soil N fertility are fully encouraged. Rhizodeposits also contribute strongly for stable organic matter in soils improving the soil structure (Pinto et al., 2021). Caddish et al. (2002) reinforced this statement and referred that nodulated roots and rhizodeposits may be protected from mineralization then playing a more important role in building of soil structure rather than in soil N supply by its high C concentration.

Keywords: Aboveground tissue, improved permanent pasture, Mediterranean region, ^{15}N technique, recovered root and nodule, sweet and yellow lupines

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S3-P-02
Reduction in the use of herbicides favours nitrogen fixation efficiency in legumes

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In recent decades, the quality of agricultural soils has been seriously affected by the continuous and excessive application of pesticides. Currently, most of the organic soil contaminants are herbicides (Dorigo et al. 2007), responsible for the toxicity effects detected in some crops, including leguminous crops (Ugbe et al. 2016). Herbicides can affect the growth and yield of leguminous plants, as well as inhibit the legume-*Rhizobium* spp. symbiosis, thus causing a decrease in biological nitrogen fixation (Zaidi et al. 2005). Studies on the effect of herbicides on soil microorganisms and their beneficial functions, including biological nitrogen fixation in the legume-*Rhizobium* spp. symbiosis, are scarce. Thus, this study aims to evaluate the effects of two commonly used herbicides, pendimethalin and clethodim, on the legume-*Rhizobium* spp. symbiosis.

The effect of the above mentioned herbicides on the growth and nodulation of *Medicago sativa* and *Phaseolus vulgaris*, was studied. *Medicago sativa* plants were grown under axenic conditions in order to perform nodulation kinetics. Clethodim caused a 30% reduction in nodulation while pendimethalin totally inhibited it, producing a reduction in root elongation and plant weight of 60%, as well. To evaluate the effect of the herbicides on *Phaseolus vulgaris* biological nitrogen fixation capacity, the plants were grown in pots with a mixture of soil:perlite (3:1 v/v). Pendimethalin inhibited nitrogen fixation rate of *Phaseolus vulgaris* by 44%. However, clethodim, which is specifically used against monocots, did not induce significant differences. The growth of the microsymbionts of *Medicago sativa* and *Phaseolus vulgaris* (*Sinorhizobium meliloti* and *Rhizobium tropici,* respectively*)* in the presence of both herbicides, was also analyzed by colony-forming unit (CFU) quantification. *Rhizobium tropici* growth was not significantly affected by the presence of the herbicides. However, *Sinorhizobium meliloti* showed a reduction in the number of CFU of 60-fold for both herbicides. Additionally, alterations induced by the herbicides in root exudates composition that might be interfering symbiosis establishment, will be discussed.

To conclude, herbicides application reduced the legumes capacity to fix nitrogen. Also, plant and bacterial growth was limited by the presence of herbicides. Thus, a reduction in the use of herbicides in crops should be addressed, with organic farming being an alternative to the intensive use of pesticides and favoring a state of natural fertilization of the soil through greater efficiency of leguminous crops.

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S3-P-03
Can bacterial strains fight plant pathogenic fungi? Bacteria as biocontrol agents

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Fungi constitute the largest number of plant pathogens and are responsible for a range of serious plant diseases. These diseases are a major threat to crop production and cause severe economic losses. Many of these pathogens are controlled by chemical products that have a negative impact on the environment. Others, are controlled through the use of resistant varieties, but the success of this strategy is highly dependent on the fungus virulence and, in some cases, by a partial expression of resistance.

Magnaporthiopsis maydis causes late wilt in maize plants, which can lead to severe economic losses, with 80–100% infection and total yield loss reported when heavily infested fields were planted with sensitive maize hybrids. Maize grain production has increased more than eight-fold in the past century, and by 2050, it is estimated that it will contribute more than half of the increased demand for cereals. So, a disease with such a high influence in productivity will have a huge impact in food security and will be an obstacle to meet the need of food demand.

The fungus infects the roots at an early stage, but wilt symptoms usually develop when the plants approach the flowering stage, approximately 60 days after sowing. Then the lower stem becomes dry, and has a hollow and shrunken appearance. Late wilt is frequently associated with infection by secondary plant parasitic fungi causing the stem symptoms to become more severe and even higher losses in crop production.

There is an urgency in effective alternatives to mitigate the effects of late wilt and biocontrol emerges as an environmental sustainable alternative. This approach was followed in the present study, by isolating bacteria from the roots of symptomatic plants with different degrees of infection. The antifungal ability of bacterial isolates delivered 8 strains able to inhibit the fungus over 50% and some of them were able to work not only against *M. maydis* but also against *Fusarium spp*.. Additionally, some of these bacteria were also able to promote plant growth and fitness. With the aim to potentiate of developing a successful methodology to minimize the effects of the fungi, the most promising bacterial isolates can be tested in different combinations helping to reduce de effects of the disease and to reduce the economic losses.

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S3-P-04
Response of soil microbiological activity and plant-beneficial microorganisms to the introduction of cover crops in intensive horticultural production systems

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Horticultural production for industry in the Portuguese region of Ribatejo is mostly based on intensive monoculture systems with high technical intervention, resulting in soil biodiversity imbalances, loss of fertility, and progressive degradation. Agricultural intensification is often associated with decreases in the abundance and diversity of functional groups in the soil microbiota, facilitating the emergence of soil-borne diseases and compelling producers to the excessive use of phytopharmaceuticals and synthetic fertilizers. Therefore, there is an urgent need to adopt alternative strategies for more sustainable production systems.

Cover crops may provide a wide range of environmental benefits and have been considered as a valuable tool to improve the sustainability of agricultural systems. In intensive horticultural production, the introduction of cover crops may contribute to improving the soil status and to mitigate the consequences of soil degradation. The present work describes the evaluation of soil microbial activity and plant beneficial microorganisms in two field trials in Ribatejo, where different cover crops were installed in the fall-winter period, preceding the main crop of the agricultural year (tomato, potato or maize): biodiverse mixture of legumes and grasses, including clovers inoculated with rhizobia; annual ryegrass (*Lolium multiflorum*); and forage turnip (*Raphanus sativus*) for biofumigation. Control plots without cover crops were maintained in both fields. Samples of rhizospheric soil from end-of-cycle plants were collected and the following indicators were evaluated: soil enzyme activities (dehydrogenase, alkaline phosphatase, β-glucosidase), total culturable bacteria, symbiotic nitrogen-fixing bacteria (rhizobia), free-living nitrogen-fixing bacteria, phosphate-solubilizing bacteria and microorganisms with phytostimulating activity.

The results showed a general increase in soil enzyme activities when using the biodiverse mixture of legumes and grasses or annual ryegrass as cover crops, relative to soils without cover. This increase was particularly accentuated in dehydrogenase activity, which represents the metabolic activity of soil microorganisms. Cover crops also positively influenced several groups of plant beneficial microorganisms, including phosphate solubilizing bacteria and microorganisms with phytostimulating activity. The evaluation of the rhizobial abundance in these soils revealed very low and ineffective native populations. However, the abundance and symbiotic effectiveness of rhizobia in the soil increased considerably following the introduction of the mixture of legumes and grasses, in which the clovers had been inoculated with rhizobia, showing the importance of introducing inoculated legumes in these soils. Free-living (non-symbiotic) nitrogen-fixing bacteria were naturally abundant in these soils and, as with total bacteria, were not affected by the introduction of cover crops. In conclusion, the obtained results indicated a tendency for the increase of soil microbiological activity and beneficial microorganisms with cover crops, especially with the biodiverse mixture of legumes and grasses and annual ryegrass. The evaluations are now proceeding in new field trials testing biodiverse cover mixtures based on selected ecotypes, optimally adapted to the cultural systems and to climate change.

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S3-P-05
Metal tolerance and accumulation in white lupin (*Lupinus albus*)

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Heavy metal contamination of soils is increasing mainly due to anthropogenic activities. To address this problem, it seems essential to find useful phytoremediation tools that are cost-effective and environmentally friendly. The aim of this work was to assess the metal tolerance of white lupin (*Lupinus albus*) and its potential for phytoremediation strategies. The tolerance to different metals of *L. albus* cv. Orden Dorado seedlings was evaluated by their relative root growth after being exposed to heavy and light metals and metalloids (Pb, Cr, Cu, Zn, Al and As). The effect of these contaminants on the antioxidant metabolism (superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase) and protein carbonylation was estimated, and metal accumulation in roots was determined by ICP-OES. *L. albus* seedlings showed tolerance to metals that was highest for aluminium and decreased in the following order: Al > Zn > Pb > As > Cu > Cr. Most antioxidant enzyme activities and protein carbonylation increased under metal stress. Finally, metal accumulation in the roots was highest for zinc and decreased in the following order: Zn > Pb > Al > Cu > As ≈ Cr.

The metal tolerance and accumulation capacity of *L. albus* cv. Orden Dorado plants inoculated with the Hg-resistant *Bradyrhizobium canariense* L-7AH strain were also investigated. Previous results from our group suggested that inoculation with *B. canariense* L-7AH conferred *L. albus* plants tolerance to mercury (Quiñones *et al*., 2013). Furthermore, those plant-*rhizobium* pairs were capable of accumulating extremely high amounts of Hg in their underground organs, particularly the cluster roots (Quiñones *et al*., 2021). *B. canariense* L-7AH is relatively tolerant to other metals besides Hg. Therefore, inoculation with this strain might have a positive effect on the plant metal tolerance. Plants were grown under cluster root-promoting conditions that consisted on limiting the availability of P during the first weeks of development, and were exposed to the above-mentioned metals. Six weeks later plants were collected, and several morphological and physiological parameters of the above and underground parts were measured. All plants presented nodules and cluster roots. Metals did not cause significant negative effects on most parameters studied, except for As which significantly affected plant development. Our results suggest that nodulated *L. albus* plants might function as a phytoremediation tool in metal-contaminated soils through rhizostabilization.

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S3-P-06
Gene duplication and enhanced expression enable rhizobia tolerance in mercury-contaminated soils

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Mercury (Hg) is extremely toxic for all living organisms, and Hg contamination is increasing worldwide in both wild ecosystems and agricultural soils due to natural processes, but mostly to anthropic activities. It has been shown that Hg-tolerant symbiotic rhizobia have the potential to increase legume metal tolerance. In plants, gene duplication is a common phenomenon in hypertolerance to heavy metals but less is known about the role of gene duplication for tolerance to toxic metals in microbes. Gene expression levels might also contribute to increased tolerance. In order to investigate the molecular mechanisms underlying Hg tolerance in rhizobia, we isolated several strains of *Ensifer medicae* and *Rhizobium leguminosarum* bv. *trifolii* from severely Hg-contaminated soils. We assembled and annotated the genomes of twelve rhizobia strains that showed wide variation in tolerance to Hg, and found structural variations in mercury reductase (*merA*) and alkylmercury lyase (*merB*), which are involved in Hg detoxification, and entire *mer* operons that were associated with the most Hg-tolerant strains. Genes in the *mer* operons and duplicated *merA* copies throughout the genomes showed significantly higher gene expression in the tolerant vs. less tolerant rhizobia strains. In the most tolerant *E. medicae* strain, a whole *mer* operon was located in a large additional 71-kb plasmid, which was not present in any other strain. Plasmid transfer to a non-tolerant strain arises as a possibility to obtain increased Hg tolerance.

Acknowledgements

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S3-P-07
Characterization of leguminous root nodule microbiome

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Soil microorganisms have an important role in the maintenance of soil functions in natural and managed agro-forestry ecosystems. Therefore, evaluating a particular group of microorganisms, such as legume root nodule bacteria/nitrogen fixing bacteria, can be a good contribution to understanding the extent to which the soils are degraded in the context of climate changes and the increase of pathogens. Likewise, the loss of vitality and the consequent death of cork and holm oaks in *Montado* areas have been associated to the frequent presence of *Phytophthora cinnamomi*. In this way, the study of symbiotic relationships between legumes and bacteria might provide a strategy to develop a more sustainable *Montado* (Videira e Castro *et al*., 2019). The aim of this work was to use symbiotic relations between *Trifolium subterraneum* and root nodule bacteria and evaluate their microbiome, which could be useful to reduce *Phytophthora* activity, and also could improve nutrients, by phosphate solubilization, in soils of *Montado*. One hundred and twenty (120) strains isolated from root nodule of *T. subterraneum* inoculated with different soils from *Montado* area (Barrancos, Portugal) were characterized for antagonism to *Phytophthora*, cellulase activity and phosphate solubilization through *in vitro* tests. Several approaches were developed using *T. subterraneum* as trap host. These essays were carried out in controlled conditions and plants were inoculated with strains previously characterized. Some of these tests were performed in presence of *P. cinnamomi* to evaluate the degree of antagonism of the root nodule bacteria tested. After this selection a consortium containing some of these root nodule bacteria was prepared and used to inoculate seeds of *T. subterraneum* in a field essay in a *Montado* area (Grândola, Portugal) where the presence of *P. cinnamomi* was previously detected. Two years after, the nodule bacteria were reisolated and characterized.

The results obtained for the *in vitro* tests showed that only a small number of isolates had at least one of the tested activities distributed as follows: phosphate solubilization (6%), antagonism to *Phytophthora* (4%) and cellulase activity (17%). Despite the small number of bacteria that showed phosphorus solubilisation, this activity is considered of great importance in poor and degraded soils, due to the poor availability of this mineral. Also, the ability to degrade cellulose could be a crucial aspect in combating/reducing *Phytophthora* as it could have direct effects by degrading its cell walls. Tests carried out in a controlled environment with *T. subterraneum* and root nodule bacteria strains in the presence of *P. cinnamomi* showed cases of pathogen inhibition and allowed the selection of a set of strains (consortium), molecularly identified as *Bacillus subtilis*, *Pseudomonas moraviensis*, *Streptomyces umbrinus* and also as *Rhizobium sp*.. These selected strains have cellulase activity and phosphorus solubilisation and, in the case of *Rhizobium* sp., also nitrogen fixation. At the end of field assay, the shoot dry weight (mg/plant) and the effectiveness index of *T. subterraneum* plants inoculated with the consortium were the highest when compared with plants inoculated with a very efficient strain routinely used (positive control).

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S3-P-08
Screening for *in vitro* antifungal activity of plant beneficial rhizosphere bacteria against important forest pathogenic fungi

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Phytopathogenic fungi, including quarantine organisms, represent important phytosanitary problems for agricultural and forestry systems, causing relevant yield decreases and reducing economic revenues. Control of these agents through classical chemical methods in forests is generally of low efficiency and may represent high environmental risks for the ecosystems. Therefore, the search for environmentally friendly solutions, such as novel microbial natural biocides in order to avoid the environmental pollution from the use of synthetic pesticides, is of much relevance. Rhizosphere bacteria, e.g. colonizing the root nodules of Leguminosae, often drive beneficial impacts on plants (Soares et al. 2021), beyond being important nitrogen fixing promotors. They also may promote plant growth and protect plant health from the action of phytopathogenic agents (Videira e Castro et al. 2019) and may, therefore, have potential use for biocontrol.

In the present study, rhizospheric bacterial strains isolated from root nodules of different species of leguminous plants in Portuguese stands, were screened for antifungal activity against forest pathogenic fungi affecting *Castanea sativa*, *Eucalyptus* spp., *Pinus* spp. and *Quercus* spp. A total of 11 fungi, namely *Cryphonectria parasitica*, *Diplodia corticola*, *Diplodia mutila*, *Diplodia sapinea*, *Fusarium circinatum*, *Heterotruncatella* sp., *Neofusicocum australe*, *Neofusicocum parvum*, *Pestalotiopsis australis*, *Pestalotiopsis pini* and *Sydowia polyspora*, were used to assess the fungal growth inhibition by rhizospheric bacterial strains in a dual culture assay, i.e., using yeast mannitol agar (YMA) and potato dextrose agar (PDA) media.

Out of the 57 tested strains, 14 were able to inhibit the growth of at least one of the tested fungal isolates. Strain #2 was the most effective strain, inhibiting the growth of six fungal isolates, including *D. sapinea* and *S. polyspora*, important conifer pathogens. These results show that these strains, in particular, strain #2, may be potential biocontrol agents against fungi causing severe forest species diseases. Hence, the most effective strains were selected for additional studies, with more extended assays.

It is important to further evaluate the source (e.g. compound) that could be responsible for the antifungal activity and to evaluate *in vivo* effects on disease expression and plant growth, thus approaching field application readiness.

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S3-P-09
Nematicidal potential of genus *Bacillus* against the root-lesion nematode *Pratylenchus penetrans*

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The root-lesion nematode (RLN) *Pratylenchus penetrans* is a migratory plant-parasitic nematode (PPN) that affects economically important agricultural crops. This nematode is spread worldwide, being reported in more than 400 host plants (mostly food and feed crops) (Castillo P and Vovlas, 2007; Vicente et al., 2022). Currently there is no effective method for the RLN control. The use of chemical nematicides is almost restricted due to their collateral damages for the environment and human health. On the other hand, the use of microbial agents as potential antagonistic against *P. penetrans* is still overlooked (Kumar and Dara, 2021). In this study, we evaluated the nematicidal potential of bacteria from genus *Bacillus* (the most reported genus expressing nematicidal effect to several PPN) to control the RLN *P. penetrans,* using the bacterial lysates and filtrates. After 24h exposure to bacterial lysates, the nematode remained alive without any deleterious effect on their normal sinusoidal movement. However, in the case of bacterial filtrates, 2 strains belonging to the *Bacillus subtilis* complex (14C2 and 14C26) presented high nematicidal effect with a mortality corrected (Mc) higher than 80%. Also, in this complex, *Bacillus* sp. 14M3 recorded moderate activity (nearly 50% Mc), while *Bacillus* spp. 4809, 13C18 and 13C43 and *B. thuringiensis* 13C9 showed no nematicidal activity. Strains *Paenibacillus lautus* 14C48 and *Priestia aryabhattai* MABNR01 showed moderate activity against *P. penetrans*. Future work will focus on the characterization of the bioactive compounds present in the bacterial filtrates of strains *Bacillus* sp. 14C2 and 14C26 and their mode of action against *P. penetrans.*

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XVIII SEFIN OEIRAS '22

BeMiPlant

Beneficial Plant-Microbe Interactions

SESSION 4

Nitrogen-Fixing Systems

S4-L-01
A life in love with science. *In memoriam* Tomás Ruiz Argüeso.

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During the Covid-19 pandemics the community of Spanish researchers working on Biological Nitrogen Fixation lost one of its most signified and respected members, **Tomas Ruiz Argüeso** (Villamol 1943 - Madrid 2020). Tomás, a longtime Professor of Microbiology and, until his passing, Emeritus Professor at Universidad Politécnica de Madrid, devoted his life to the study of the symbiotic nitrogen fixation process. His research on the Rhizobium-legume system was indeed transversal, producing relevant contributions in the physiology, regulation, genetics, genomics, and ecology of this symbiosis. He was one of the founding members of SEFIN, and mentor to a great number of researchers in the field. In this meeting we want to present a tribute to his memory, to his many scientific achievements, to his never-ending scientific curiosity and pleasure from doing science, and to his friendly and close character, always ready to listen and help others. All of us having had the privilege of sharing work and life with Tomás will miss him. His memory will illuminate and accompany us for the years to come.

S4-L-02
Engineering nitrogen fixation in cereals

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Integration of nitrogen fixation traits into cereal crops requires the assembly in plant cells of active nitrogenase complex (NifHDK) encoded by transferred prokaryotic genes. The feasibility of this transgenic approach was explored by expressing two critical genes (*nifH* and *nifB*) in rice. The *nifH* gene encodes the nitrogenase Fe protein, the obligate electron donor to NifDK for the nitrogen fixation reaction. NifB catalyzes the first committed step in the biosynthesis of the FeMo-cofactor located at the active-site of the nitrogenase of MoFe protein (NifDK). Production of NifB and NifH in plants is challenging because they are iron-sulfur proteins extremely sensitive to O_2. We generated rice plants expressing either NifH or NifB in mitochondria, which should limit exposure to O_2 and provide essential [Fe-S] clusters required for activity. NifM and FdxN accessory proteins were co-expressed with NifH and NifB, respectively. Engineered rice callus and plants derived from them express soluble and stable Nif polypeptides in mitochondria. The approach of targeting highly soluble and thermostable NifB synthetic variants to mitochondria succeeded in recovering proteins that were functional in FeMo-co synthesis and nitrogenase activation when tested *in vitro*. Similarly, a synthetic variant of a thermophilic NifH incorporated endogenous [4Fe-4S] clusters, to some degree, and gained fundamental activities in the context of nitrogen fixation, including electron transfer to NifDK. The approach of targeting a highly soluble and thermostable Nif variants to mitochondria overcomes several major constraints to engineer nitrogenase in rice, and confirms that incorporation and stability of [Fe-S] clusters in nitrogenase components constitutes the next critical bottleneck to address in this process. This report represents an important step toward the expression of a complete functional Nif complex as required to achieve independent biological nitrogen fixation in cereals.

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S4-O-01
MtAtx1 is a Cu^+-chaperone involved in Symbiotic Nitrogen Fixation

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Copper is an essential micronutrient for Symbiotic Nitrogen Fixation (SNF) acting as cofactor of many of the key enzymes involved in this process. Therefore, copper insufficiency results in a diminished SNF. Once this nutrient is incorporated into the nodule cells through nodule-specific Cu^+-transporter COPT1 (Senovilla *et al.*, 2018), it must be bound to acceptor molecules, as free copper in solution catalyzes toxic Fenton-type reactions (Robinson and Winge, 2010). To prevent it, intermediate proteins, Cu^+-chaperones, that mediate copper delivery from the transporter to the acceptor proteins are needed (O'Halloran and Culotta, 2000). We analysed the transcriptome of the *M. truncatula* legume and MtAtx1 was identified as the only nodule-specific Cu^+-chaperone. MtAtx1 presents the classic Cu^+-chaperone domain in N-terminal, with the CXXC motif that coordinates copper. Moreover, confocal microscopy images located the protein in the cytosol of cells from Infection/differentiation zone of the nodule. At the subcellular level, electron microscopy revealed an association between MtAtx1 and the symbiosome and plasma membranes. A knock-out mutant line *atx1-2* showed reduced nitrogenase activity compared to WT plants. Thus, we propose MtAtx1 as the nodule-specific Cu^+- chaperone in charge of providing the cofactor to the copper proteins present in the nodule.

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S4-O-02
Differential expression of hemoglobins in nodules of the model legume *Lotus japonicus* suggests specific functions

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Hemoglobins occur in all organisms and perform multiple functions, including the transport, delivery, and scavenging of O_2 and nitric oxide. In plants, there are two types of hemoglobins: nonsymbiotic or phytoglobins (Glbs) and symbiotic, known in legumes as leghemoglobins (Lbs). Based on phylogeny and biochemical properties, Glbs can be further categorized into three classes. Class 1 Glbs occur in monocots and dicots and have high O_2 affinity; class 2 Glbs are only present in dicots and have moderate O_2 affinity; and class 3 Glbs are widespread but have lower O_2 affinities[1]. Lbs have similar O_2 affinities to class 2 Glbs, from which most of them derive. In legume nodules, Lbs provide O_2 to bacteroids, thus maintaining an optimal O_2 environment for N_2 fixation. The nodules of *Lotus japonicus* express three *Lb* genes: *LjLb1* and *LjLb2* are located in tandem on the same chromosome and have 98% sequence identity, whereas *LjLb3* shares 84% with the other two genes. In addition to the *Lb* genes, the genome of *L. japonicus* encodes two class 1 Glbs (LjGlb1-1 and LjGlb1-2), two putative class 2 Glbs (LjGlb2-1 and LjGlb2-2), and two class 3 Glbs (LjGlb3-1 and LjGlb3-2).

Here, we have studied the effect of nodule senescence on the expression of Glbs and Lbs in *L. japonicus* inoculated with *Mesorhizobium loti* strain MAFF303099. Nitrate-induced nodule senescence was analyzed by treating plants at 4 weeks post-inoculation (wpi) with 5 mM KNO_3 for two days. The expression of the three *LjLbs*, *LjGlb2-1*, and *LjGlb2-2* was downregulated after nitrate treatment. In contrast, the mRNA levels of class 1 and class 3 Glbs increased in nodules of plants treated with KNO_3. Natural senescence (aging) was studied in nodules of 2, 4, 6, 8, and 10 wpi. We observed that LjLbs show differential expression profiles during nodule development. Thus, *LjLb1* expression was highest in young nodules (2 wpi), *LjLb2* expression decreased in old nodules (8 and 10 wpi), and *LjLb3* expression did not change during nodule aging. The expression of *LjGlb2-1* and *LjGlb2-2* decreased with nodule aging and showed a similar pattern to *LjLb1*. The expression of *LjGlb3-1* decreased in old nodules (8 and 10 wpi) similarly to what was observed for *LjLb2*. However, *LjGlb1-2* expression was enhanced in mature nodules (4 and 6 wpi). Further insights into the regulation of hemoglobin genes was gained using single (*lb3*), double (*lb13* and *lb23*), and triple (*lb123*) knockout mutants of *L. japonicus* defective in Lbs, which were generated by CRISPR/Cas9. First, we phenotyped nodulated plants at 4 wpi. Mutant plants showed a delay in growth that was directly related to the number of Lbs that were lacking in nodules. Second, expression (mRNA and protein) of Lbs and Glbs was examined in the mutant nodules. Compared with the wild-type, the expression of *LjLb1* and *LjLb2* was greater in the *lb23* and *lb13* mutants, respectively, indicating that these nodules tend to compensate the total amount of Lbs to maintain N_2 fixation. Also, *LjGlb1-1* was down-regulated whereas *LjGlb3-1* was up-regulated in *lb123* nodules. Therefore, our results show differential expression for each Lb and Glb during nodule development and/or in nodules of the *lb123* mutant, pointing to distinct regulatory mechanisms and functions.

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S4-O-03
Effect of nitrogen supply and Rhizobia symbiosis in the isotopic composition of essential plant elements, nutrient content, TCA cycle activity and respiratory energy balance of *Lotus japonicus*

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There is a lack of studies unravelling the effect of legume-rhizobia interactions on plant respiratory energy efficiency. In plants, the existence of the alternative oxidase pathway (AOP) in the mETC confers metabolic flexibility by regulating the dissipation of reducing equivalents from TCA cycle, helping to maintain redox status and nutrient balance but decreasing yield of respiration. It is thought that carbon requirements of the symbiont and nitrogen transfer to the plant from nodules may affect the activities of both cytochrome oxidase pathway (COP) and AOP in plant organs for the benefit of plant yield in N poor soils. The main objective of this research was to create different plant N status by growing plants of WT *Lotus japonicus* at 5 mM and 10 mM KNO_3, and in symbiosis with *Mesorhizobium meliloti* (0 mM KNO_3). Besides, plants displaying spontaneous nodule formation (snf) mutations were grown at 1 mM KNO_3. By isotope-ratio mass spectrometry, we evaluated discrimination against ^{18}O during respiration, and $\delta^{13}C$ and $\delta^{14}N$ in plant organs to determine ATP synthesis, changes in plant C economy, and N transfer from nodules. By high-performance liquid chromatography (HPLC) and inductively coupled plasma (ICP) spectrometry, we also determined the content of NADH and nutrients as proxies of changes in TCA cycle activity and in plant nutrient economy. Our results indicated that nitrogen is vital in the modulation of respiratory metabolism, and that symbiosis improves production of ATP via COP, probably due to an incremented photosynthetic demand of symbiont for inorganic carbon and improved N status. Overall, our results shed some light into the complexity of legume-rhizobia interactions involving plant respiration and essential plant nutrients.

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S4-P-01
Iron homeostasis in nitrogen fixation: Role of glutaredoxin5

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The metalloenzyme nitrogenase requires iron-sulfur clusters (Fe-S) to catalyze the reduction of N_2 into NH_3, an essential process in biosphere. Nitrogenase assembly and metal cofactor formation in the diazotrophic bacterium *Azotobacter vinelandii* have been described in detail (Burén et al., 2020). NifU acts as molecular scaffold where Fe-S clusters destined for nitrogenase are first assembled. In this process, sulfur is delivered by NifS, a cysteine desulfurase, but the iron donor is still unknown. We hypothesize that another protein must provide iron to NifU as this element is not free, hydrated, in the cytosol as it could produce damaging free radicals in Fenton-style reactions (Winterbourn, C. C., 1995).

A pull-down assay using purified NifU and *Azotobacter vinelandii* DJ extract was performed to identify the NifU iron donor. Among all the interactors, glutarredoxin5 (Grx5) was a promising candidate for this function since other glutaredoxins have been involved in Fe-S metabolism (Couturier et al., 2015). Interaction assays between purified NifU and Grx5 were performed to confirm the pull-down result. The presence of labile Fe-S clusters in NifU or Grx5 seemed to stabilize the interaction, suggesting a possible Fe-S transfer between both proteins. To study the physiological role of Grx5 under diazotrophic conditions, a *grx5* mutant was produced in *A. vinelandii*. Nitrogenase activity assays indicated that the *grx5* mutant fixes less nitrogen than wild type, evidencing that Grx5 is required for an optimal nitrogenase activity.

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S4-P-02
Azotobacter vinelandii scaffold protein NifU transfers iron to NifQ as part of the iron-molybdenum cofactor biosynthesis pathway for nitrogenase

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Azotobacter vinelandii molybdenum-dependent nitrogenase obtains molybdenum from NifQ, a monomeric iron-sulfur molybdoprotein. This protein requires of a preexisting [Fe-S] cluster to form a [$MoFe_3S_4$] group to serve as specific donor during nitrogenase cofactor biosynthesis. Here, we show biochemical evidence for NifU being the donor of the [Fe-S] cluster. Protein-protein interaction studies using apo-NifQ and as-isolated NifU demonstrated the interaction between both proteins which is only effective when NifQ is unoccupied by its [Fe-S] cluster. The apo-NifQ iron content increased after the incubation with as-isolated NifU, reaching similar levels to holo-NifQ after the interaction between apo-NifQ and NifU with reconstituted transient [Fe_4-S_4] groups. These results also indicate the necessity of co-expressing NifU together with NifQ in the pathway to provide molybdenum for the biosynthesis of nitrogenase in engineered nitrogen-fixing plants.

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S4-P-03
Role of MtYSL3 and MtYSL7 in symbiotic nitrogen fixation

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Yellow Stripe-Like (YSL) proteins are a family of transporters typically associated with transition metal homeostasis. *Medicago truncatula* genome encodes eight members of this family (MtYSL1-8). Two of them, MtYSL3 and MtYSL7, play an important role in symbiotic nitrogen fixation as indicated by the severe reduction of nitrogenase activity observed in *Tnt1* insertional mutants. MtYSL3 is expressed in root and nodule endodermis and in the nodule cortical cells. Its mutation leads to reduced iron content in nodules, as well as altered iron and zinc distribution. X-ray fluorescence images suggest that these elements are retained in the nodule vasculature, what is consistent with a role on metal delivery to nodules. MtYSL7 is also highly expressed in nodules, with an expression pattern similar to MtYSL3. *Tnt1 ysl7* mutants grew less than the wild type plants both in symbiotic and in non-symbiotic conditions. Unlike *ysl3* mutants, iron concentration in *ysl7* nodules was higher than in wild type controls, and no altered iron distribution was observed in these nodules. Moreover, MtYSL7 does not transport iron/iron chelates when produced in yeast, but short peptides. This, together with the up-regulation of iron-deficiency genes in *ysl7* roots, would indicates a role in iron sufficiency signalling, rather than direct metal supply to nodules.

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S4-P-04
Symbiotic characterization of chickpea germplasm and seed composition

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Chickpea (*Cicer arietinum* L.) is one of the earliest cultivated legumes, belongs to the family Fabaceae, (subfamily Faboideae). Remains of this pulse from the Middle East have been found that are around 7,500-9,000 years ago. Today, chickpea is the world´s second most widely grown after soybean and its cultivation is well adapted to the climate and agronomic features of the Mediterranean basin. Domesticated chickpea varieties belong to two main groups called desi and kabuli with distinguishing differences in seed color and size, and flower color.
Legumes breeding programs, with few exceptions, has been carried out disregard FBN capacity of these plants, thus Germplasm accessions and /or advanced varieties have not been characterized symbiotically.
Plant inoculation tests have been carried out with specific mesorhizobia on selected chickpea varieties or RILs (Recombinant Inbreed Lines) from IFAPA-Centro Alameda del Obispo (Córdoba, Spain), plus a variety from Italy (Cere nero, black color). Most of the varieties tested belong to kabuli group and are non-pigmented.
All varieties did form nodules regardless the effectiveness on nitrogen fixation, thus some combinations were defined as ineffective while the desi ferruginous-coloured cultivar (5-RIL-33) outperform the other genotypes in all symbiotic features. Previous reports have shown the symbiotic advantages of colored seeds on chickpea and other legumes as Phaseolus vulgaris and *Vigna subterranea* (Hungria & Phillips 1993; Puozaa et al. 2021) tightly related with phenolic composition of seeds. Our preliminary results shown that colored seeds present exclusively some flavonoids that would be correlated with its superior nitrogen fixation capacity, although more chickpea genotypes should be tested.

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S4-P-05
Sinorhizobium fredii HH103 effectively nodulates *Robinia pseudoacacia*, a legume tree able to form indeterminate nodules

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Robinia pseudoacacia L. (Fabaceae) (also called "black locust") is cultivated in many different parts of the world. This legume tree is widely used for timber, fodder, conservation of soils, attenuation of soil erosion, and rehabilitation of mining land and degraded industrial areas (1). It is also present as an ornamental tree in city streets, such as Seville.

Previous reports have shown that some *Rhizobium spp.* (1) and *Mesorhizobium* (2) strains are able to nodulate this legume. We have investigated whether *Sinorhizobium fredii*, a broad host-range rhizobial strain, is able to induce the formation of nitrogen-fixing nodules on *R. pseudoacacia* roots. A spontaneous rifampicin-resistant derivative of *S. fredii* HH103 strain (termed HH103 Rif-r) was used as inoculant. All the plant tests were carried out in a plant-growth chamber with a photoperiod of 16 h light/day and a temperature ranging from 26°C (light) to 18°C (dark).

In a preliminary experiment, *R. pseudoacacia* seedlings inoculated with *S. fredii* HH103 Rif-r formed nodules. Bacteria isolated from a *R. pseudoacacia* nodule induced by inoculation with *S. fredii* HH103 Rif-r was termed *S. fredii* HH103-Rsp.

S. fredii HH103 Rif-r and *S. fredii* HH103-Rsp were used as inoculants of *R. pseudoacacia* seedlings in a subsequent experiment. Two different *S. fredii* HH103 Rif-r mutants, SVQ548 and SVQ533, were also included in this nodulation assay. SVQ548 carries a mutation in the *nolR* gene, a transcriptional regulator that affect the production of different symbiotic signals, such as Nodulation factors (Nod-factors), the Type Three Secretion System (T3SS) and surface exopolysaccharides (EPS). SVQ533 carries a mutation in the *tssI* gene, which is the transcriptional regulator of genes involved in the formation of the T3SS apparatus and the proteins (called Nops) secreted through it. López-Baena et al (2016) is a review of *S. fredii* symbiotic signals (3).

Nodules induced by *S. fredii* HH103 Rif-r, *S. fredii* HH103-Rsp and SVQ548 were spherical or cylindrical 7 weeks after inoculation. However, at 12 weeks after inoculation most of the nodules were lobulated, showing the typical external morphology of indeterminated nodules. Longitudinal sections of nodules induced by these three inoculants showed internal, pink-coloured tissues. Plant-top dry-weight of *S. pseudoacacia* plants inoculated with *S. fredii* HH103 Rif-r, *S. fredii* HH103-Rsp and SVQ548 were higher than that of uninoculated plants, a clear indication that the nodules formed contributed to plant growth. *S. pseudoacacia* seedlings inoculated with mutant SVQ533 were non nodulated 7 weeks after inoculation. Few nodules were observed in some plants at 12 weeks post inoculation, suggesting that disruption of the T3SS apparatus, and the concomitant elimination of Nops secretion, reduces bacterial capacity to nodulate this legume.

A new larger plant test is being set to verify the results mentioned above. We expect to present new data in our poster presentation.

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S4-P-06
H_2O_2 production through polyamines oxidation modulate the symbiotic signaling in *Medicago truncatula*

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There are evidence supporting the implication of H_2O_2 in the early symbiosis events in legumes, including the recognition of the partners and the infection and root-nodule organogenesis (Andrio et al. 2013). One of the cellular sources of H_2O_2 is the oxidation of polyamines (PAs) by polyamine oxidase (PAO). PAs putrescine (Put), spermidine (Spd) and spermine (Spm) are ubiquitous polycationic amines found in all living cells and described as a new class of plant growth regulator (Torrigiani 1996). The multifunctional role of PAs is related with the production of H_2O_2, crucial in the regulation of plant defense responses as well as the symbiotic interactions, which is consistent with the existence of diverse PAO isoforms with tissue specific characteristics, and regulatory effect on H_2O_2 production (Angelini et al. 2010).
The model legume *Medicago truncatula* contains three *MtPAO* genes (*MtPAO1* to *MtPAO3*) with different spatial expression patterns. To address the implication of *MtPAOs* in the *M. truncatula-Sinorhizobium meliloti* symbiosis, we studied the symbiotic phenotype of knock-down mutants of *MtPAO1* and *MtPAO3*, both expressed in root nodules. We found differences in the activation of the symbiotic signaling pathway between both mutants, with an increment in the nodule number and the symbiotic gene responses to nod factors in *MtPAO3* mutants, whereas in the *MtPAO1* mutants, a significant reduction in the nodulation was detected. These results suggest the participation of different PAOs in the symbiotic and defensive responses induced during the early nodulation events.

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S4-P-07
Functional analysis of a host dependent metal transporter system in the *Rhizobium*-legume symbiosis

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Biological nitrogen fixation due to the symbiotic interaction between legumes and *Rhizobium* is a sustainable alternative to the use of nitrogen fertilizers reducing its costs and improving soil fertility. The establishment of *Rhizobium*-legume symbiosis is the result of sophisticated plant- and bacteria-dependent mechanisms required to adjust the behaviour of both partners that lead to the formation of legume nodules where rhizobia is differentiated to bacteroid, the symbiotic nitrogen fixing form of rhizobia. Proteomic comparative analysis of bacteroids induced by *Rhizobium leguminosarum* bv. viciae (Rlv) UPM791 from pea and lentil nodules showed that the expression of over 100 proteins is dependent on the legume host (Durán *et al*., 2021) indicating that the host induces in rhizobia specific responses that might define symbiotic performance. A metal-binding protein (RLV_3444), a component of the ABC transporter system RLV_3442-3444 overexpressed in pea bacteroids, was identified, suggesting that the provision of some metal(s) to the bacteroid is more restrictive in the *Rhizobium*-pea symbiosis. The objective of this work is to study the functional role of RLV_3442-3444 metal transporter system in the *Rhizobium*-legume symbiosis.

RLV_3444 is highly conserved in *R. leguminosarum* with a 95-99% identity to metal binding proteins from other rhizobial strains and species. Structural modelling and alignment have revealed that this protein shows a high similarity with zinc-, manganese- or iron-binding proteins. RLV_3444 contains three histidine residues (H62, H127 and H193) highly conserved in homologues to ZnuA, the substrate-binding protein of the high affinity zinc transporter system ZnuABC previously described in *Escherichia coli* (Yatsuniyk *et al*., 2008). In addition, zinc concentration in pea bacteroids induced by a mutant strain defective in the transport system diminished in comparison with the wild type. These results suggest that RLV_3442-3444 might be involved in zinc import into the bacteroid.

Functional analysis of RLV_3442-3444 under free-living and symbiotic conditions has shown that RLV_3444 replaces the role of ZnuA under zinc limiting conditions. The defective growth phenotype of bacterial cultures of RLV_3444/ZnuA double mutant strain was complemented by supplementing the medium with zinc but not with other metals such as manganese or iron. Analysis of transcriptional *gusA* fusions to the DNA region upstream of the operon encoding RLV_3442-3444 has demonstrated that the transporter metal system is expressed under zinc- depleted conditions and repressed in the presence of this metal. The regulatory region has been determined by the use of transcriptional reporter gene fusions to truncated forms of the DNA region upstream to the transporter gene operon.

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S4-P-08
Study of the role of stress response protein sHSP_252 in *Rhizobium*-legume symbiosis

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Rhizobium leguminosarum bv. viciae (*Rlv*) is an endosymbiont of legume plants of agronomic importance such as pea (*Pisum sativum*) and lentil (*Lens culinaris*). This symbiotic interaction leads to the formation of new structures in legume roots, the nodules where rhizobia are converted into its symbiotic form, the bacteroid, that reduces atmospheric nitrogen into ammonia which is exported to the plant. During the establishment of the symbiosis, rhizobia are exposed to hostile physical and chemical micro-environments (NCR peptides, microaerobiosis or oxidative burst) to which must be adapted to obtain effective symbiosis. Comparative proteomic analysis in pea and lentil bacteroids induced by *Rlv* UPM791 strain revealed about 100 proteins with host-dependent expression (Durán *et. al*., 2020). Among these differentially expressed proteins, small heat shock proteins (sHSPs) were identified, stress response proteins which act as chaperones stabilising partially denatured proteins. Unlike in multicellular eukaryotes, most bacteria contain one or two sHSP. In contrast, (brady)rhizobia contain 4 to 8 members of this group, suggesting the potential relevance of these proteins in the symbiosis.

The aim of this work is to study the functional role of sHSP_252, a stress response protein overexpressed in pea bacteroids, in *Rhizobium*-legume symbiosis. The results obtained indicate that sHSP_252 is required to reach maximum levels of fixed nitrogen in pea plants. Promoter region of *sHsp_252* contains two anaeroboxes, and regulation analysis by gene promoter-*gusA* fusions has shown that *sHsp_252* is expressed under microaerobic conditions in a FnrN-dependent manner. In addition, controlled induction experiments indicated that sHsp_252 is able to protect *Rlv* exposed to oxidative stress (H_2O_2). Affinity chromatography studies from microaerobic cultures and bacteroids induced by *Rlv* strain expressing an StrepTag-labelled variant of sHSP_252 have indicated that the protein is co-purified with multiple protein targets. Additionally, sHSP_252 has been localised in both soluble and membrane fractions. Further analysis will identify possible copurified sHSP_252 targets.

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S4-P-09
The *Rhizobium tropici* CIAT 899 NodD2 protein promotes symbiosis and extends rhizobial nodulation range by constitutive nodulation factor synthesis

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In the symbiotic associations between rhizobia and legumes, the NodD regulators orchestrate the transcription of the specific nodulation genes. This set of genes is involved in the synthesis of nodulation factors, which are responsible for initiating the nodulation process (Hassan and Mathesius, 2012). *Rhizobium tropici* CIAT 899 is the most successful symbiont of *Phaseolus vulgaris* and can nodulate a variety of legumes. Among the five NodD regulators present in this rhizobium, only NodD1 and NodD2 seem to have a role in the symbiotic process (del Cerro et al., 2017). However, the individual role of each NodD in the absence of the other proteins has remained elusive.

The *nodD2* gene of CIAT 899 does not require activation by inducers to promote the synthesis of nodulation factors. In fact, a CIAT 899 strain overexpressing *nodD2*—but lacking all additional *nod* genes—can nodulate three different legumes as efficiently as wild-type. Interestingly, CIAT 899 NodD2-mediated gain of nodulation can be extended to another rhizobial species, since its overproduction in *Sinorhizobium fredii* HH103 not only increases the number of nitrogen-fixing nodules in two host legumes but also results in nodule development in incompatible legumes (Ayala-Garcia et al., 2022). These findings potentially open exciting opportunities to develop rhizobial inoculants and increase legume crop production.

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S4-P-10
Looking inside *Acacia longifolia* root nodules: structure and microbial diversity

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Australian acacia species are frequently found in introduced range and 24 are considered invasive (Richardson *et al.*, 2015). *Acacia longifolia* is an aggressive invader, particularly in Portugal, dispersed mostly in coastal habitats, being a threat for biodiversity. This species is an ecosystem-engineer due to the remarkable alterations on the belowground, including soil composition and nutrient and water cycles (e.g., Marchante *et al.*, 2008). The ability to adapt fast to environmental changes renders this species an interesting plant to consider as a case study of invasive success. As a legume, *A. longifolia* establishes symbioses with soil bacteria, including rhizobia and non-rhizobia, and these relationships are advantageous for invasion (e.g., Jesus *et al.*, 2020). The symbiosis develops inside *de novo* differentiated structures (root nodules), where bacteria and plant exchange fixed nitrogen for carbohydrates. In this study, we selected a maritime pine (*Pinus pinaster* Aiton) forest invaded by *A. longifolia* in Mira (Aveiro, Portugal) and we collected root nodules from young plants. We addressed root nodule structure through histological studies following formaldehyde-glutaraldehyde fixation, embedding in paraplast and staining with Toluidine Blue, Lugol or Löffler solution. Also, we performed Next Generation Sequencing using Oxford Nanopore Technology, targeting 16S rRNA gene, to identify the bacteria present in the root nodule community. A preliminary assessment on fungal diversity inside nodules was performed using the internal transcribed spacer region. We found that nodules are of standard indeterminate-type and are divided in four different zones: meristematic zone, infection zone, nitrogen-fixing zone, and senescence zone. Bacteria were present inside the cells, filling them. A higher number of infected cells was observed in the nitrogen-fixing zone along with an accumulation of starch granules in the adjacent uninfected cells, suggesting that starch is the carbon source for bacteria development. Regarding bacterial diversity, we found a dominance of *Bradyrhizobium* followed by *Paraburkholderia*, both included in rhizobia group, and *Massilia* as a third bacterial partner (non-rhizobia). Yeasts were observed following microscopic observation of the semi-thins sections. The preliminary assessment on fungal diversity highlighted the presence of *Coniochaeta* as the main genus present followed by *Mucor* and *Alternaria*. This diversity in root nodules raise the question to which extent nodulation is just restricted to nitrogen fixation, suggesting different bacterial roles as well as a tripartite symbiosis including fungal partners. Such diverse nodule microbiota found in *A. longifolia* could contribute to explain this species success, particularly under climate change scenario.

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S4-P-11
Persulfidation of plant and bacteroid proteins is involved in legume nodule development and senescence

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Legumes establish symbiosis with soil rhizobia forming root nodules that fix atmospheric nitrogen. This symbiotic nitrogen fixation has a major interest in agriculture. The interaction between legumes and rhizobia needs sophisticated signaling networks involved in bacterial recognition, root colonization, and regulation of nodule metabolism and senescence. The central role in nodule biology of reactive oxygen species (ROS), such superoxide and peroxides, and reactive nitrogen species (RNS), such as nitric oxide and *S*-nitrosothiols, is broadly acknowledged (Minguillón *et al.*, 2022). Recently, hydrogen sulfide (H_2S) and other reactive sulfur species (RSS) have emerged as novel signaling molecules in animals and plants with important potential functions in developmental and stress responses (Gotor *et al.*, 2019). A major mechanism by which ROS, RNS, and RSS fulfil their signaling role is the post-translational modification of proteins (Matamoros and Becana, 2021). To identify possible functions of H_2S in nodule development and senescence we used the tag-switch method to analyze quantitative changes in the persulfidation profile of common bean (*Phaseolus vulgaris*) nodules. We identified 967 proteins of the host cells and 409 proteins of bacteroids with altered levels of persulfidation at different developmental stages. The proteomic analysis suggests that persulfidation plays a major regulatory role in plant and bacteroid metabolism and senescence. In addition, we investigated the effect of a H_2S donor on several proteins involved in ROS and RNS homeostasis, including iron superoxide dismutase, glutathione peroxidase, class 3 non-symbiotic hemoglobin, and the enzymes of the ascorbate-glutathione pathway. The results obtained using nodule extracts and recombinant proteins suggest that H_2S and persulfidation protect redox-sensitive enzymes from oxidative modifications that may cause enzyme inactivation. They also support that the general decrease of persulfidation levels observed in plant proteins of aging nodules is a mechanism that causes the disruption of redox homeostasis leading to senescence.

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S4-P-12
Characterization and expression profile of the "Amidoxime Reducing Component" enzymes (LjARC1 and LjARC2) of the model legume *Lotus japonicus*

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The "Amidoxime Reducing Component" (ARC) is an enzyme discovered in humans that catalyzes the reduction of *N*-hydroxylated compounds to the corresponding amines, and its activity is usually assayed with benzamidoxime as artificial substrate (Sparacino-Watkins *et al.,* 2014). A surge of interest on the enzyme in the plant kingdom was raised by the finding of Chamizo-Ampudia *et al.* (2016) that the single ARC of the unicellular green alga *Chlamydomonas reinhardtii* is able to utilize NADH, in combination with nitrate reductase, to reduce nitrite to nitric oxide (NO). In fact, these authors renamed the enzyme as NO-forming nitrite reductase (NOFNiR). *Arabidopsis thaliana* has two *ARC* genes located in different chromosomes but it has been very recently concluded that none of them encodes a NOFNiR that is functional at physiological nitrite concentrations (Maiber *et al.*, 2022).

The model legume *Lotus japonicus* also has two *ARC* genes, although they are arranged in tandem on the same chromosome. The corresponding proteins, LjARC1 and LjARC2, share high amino acid sequence identity (74%) and similarity (84%). Our objective was to investigate whether LjARC1 and/or LjARC2 can act as NOFNiR. To this end, we have first produced the proteins in recombinant form and shown that they are functional in reducing the artificial substrate benzamidoxime to benzamidine with dithionite as electron donor. We then showed that nitrite competes with benzamidoxime in the assay of LjARC1 and LjARC2, indicating that nitrite can be a substrate and suggesting that the enzymes might be NOFNiRs. Preliminary measurements of NO production with the NO analyzer showed that the enzymes generate in vitro NO from nitrite using dithionite as reductant at physiological pH and low nitrite concentrations.

We have also examined the expression profiles (mRNA levels) of *LjARC1* and *LjARC2* in plant tissues. The expression of *LjARC2* was higher than that of *LjARC1* in all plant tissues. For both genes, mRNA levels were highest in pods, then in flowers and leaves, and finally in roots and nodules. Likewise, we have developed a method based on HPLC to assay ARC activity (LjARC1 + LjARC2) in plant tissues. As occurs for mRNAs, the highest activity was found in pods, followed by flowers and leaves. Experiments are under way to conclusively demonstrate that LjARCs are capable of generating NO from nitrite using natural reductants (NADH and NADPH) in combination with purified nitrate reductase.

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S4-P-13
Screening of suitable Nif proteins for nitrogenase engineering in eukaryotes

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Engineering functional molybdenum nitrogenase into plants requires a minimal set of 6 Nif polypeptides, the catalytic NifHDK module and the iron-molybdenum cofactor (FeMo-co) biosynthesis NifENB module. NifB is key to FeMo-co biosynthesis as it catalyzes the synthesis of its precursor NifB-co, which is also the precursor of FeV-co and FeFe-co from the alternative nitrogenases. Initial attempts of expressing NifB from model diazotrophs in heterologous eukaryotic systems rendered mostly insoluble protein[1], while other works proved that Nif proteins from archaeal origin performed better[2,3]. Recently, yeast co-transformed with NifB and NifU, NifS and FdxN for the biosynthesis of [Fe-S] clusters produced NifB-co *in vivo*[2].

In this work, 30 *nifB* genes[2] were transiently expressed into *Nicotiana benthamiana* and targeted to either mitochondria or chloroplasts together with NifU, NifS and FdxN. A screening based on NifB solubility and level of accumulation was performed to select the most promising variants of the library. *Methanocaldococcus infernus, Methanosarcina acetivorans* and *Methanothermobacter thermautotrophicus* NifB were selected and purified from either *N. benthamiana* mitochondria or chloroplasts. Purified *N. benthamiana* NifB proteins were tested in the *in vitro* FeMo-co synthesis and nitrogenase activation assay, which permits to validate the activity of each individual protein in the nitrogenase maturation pathway. All selected *N. benthamiana* NifB proteins were competent in NifB-co synthesis, rendering nitrogenase activity values similar to NifB purified from diazotrophic bacteria.

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S4-P-14
Novel enzymes involved in N_2O emission by *Rhizobium etli* – common bean symbiosis

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Nitrous oxide (N_2O) is a powerful greenhouse gas due to its high chemical stability and great radiative potential. More than 60% of total N_2O emissions to the atmosphere come from agricultural soils mainly because of the excessive application of nitrogen fertilizers. Ammonia (NH_4^+) and nitrate (NO_3^-) are both substrates for nitrification and denitrification, the two main microbial processes that contribute to N_2O emissions from soils. Biological Nitrogen Fixation by rhizobia-legume symbiosis is proposed as an environmentally friendly strategy to decrease dependence on nitrogen fertilizers and, therefore, mitigate N_2O emission. However, rhizobia inside legume nodules can also perform denitrification. This process consists in a sequential reduction of NO_3^- to nitrite (NO_2^-), nitric oxide (NO), N_2O and molecular nitrogen (N_2), four reactions that are catalyzed by the periplasmic (Nap) or membrane bound (Nar) nitrate reductases, nitrite reductases (NirK/*cd*1Nir), nitric oxide reductases (cNor, qNor/Cu_ANor), and nitrous oxide reductase (Nos) encoded by *nap*/*nar*, *nir*, *nor* and *nos* genes, respectively. *Rhizobium etli* CFN42, the microsymbiont of common bean, is unable to respire nitrate under anoxic conditions and perform a complete denitrification pathway. This bacterium lacks the *nap*, *nar* and *nos* genes but contains genes encoding NirK and cNor. Despite lacking the *nap* /*nar* genes, *R. etli* is able to produce N_2O under free living conditions when cultured microoxically with NO_3^- as the only nitrogen source, thanks to the coupling of an assimilatory nitrate reductase (NarB) with the NirK and cNor denitrification enzymes (1). In this work, we have identified a NarK protein in *R. etli* genome and we have demonstrated that it is involved in nitrite extrusion being the link between the nitrate assimilation and denitrification pathways. Furthermore, our results demonstrate for the first time that common bean nodules from plants inoculated with *R. etli* and exposed to NO_3^- emit N_2O. The involvement of NarB, NirK, cNor and NarK in N_2O emissions as well as in bacteroidal nitrate-, nitrite- and nitric oxide reductase activities has also been established. By analysing Nitrosyl–leghemoglobin complexes in whole intact nodules using Electron Paramagnetic Resonance (EPR) spectroscopy, we have demonstrated the involvement of NarB and NorC in NO production and consumption in the nodules.

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XVIII SEFIN OEIRAS '22

BeMiPlant

Beneficial Plant-Microbe Interactions

SESSION 5

PGPR, Mycorrhizae and Microbial Endophytes

S5-L-01
PGPR and bacterial endophytes: Phosphate solubilizing bacteria - where to find?

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The continuous growth of world population has put enormous pressure to increase food production and to achieve food security. The green revolution that took place from the 1960s onwards allowed to sustain the population increase (Briggs, 2009) at the expense of agricultural practices based on heavy fertilization and agrochemicals with negative impact on the environment. Climate change and environment degradation called into question these practices, evidencing the urgency for the adoption of new and more sustainable practices. Plant Growth Promoting Bacteria (PGPB) play important roles in survival and health of plants, providing plants with nutrients, protecting them from pathogens and helping them overcome abiotic stresses, boosting plant productivity sustainably (Chandran et al., 2021). One of the most limiting nutrients of plant growth is phosphorous (P). The availability of the P present in the soil is low due to fixation in insoluble forms of the phosphate fertilizers applied in soils. Soil microorganisms play a central role in the biogeochemical cycling of P, converting unavailable P to available forms, and enabling plants to uptake P (Bhattacharya et al., 2019). Using results from our laboratory, this presentation addresses factors influencing the relative abundance of P-solubilizing bacteria: influence of climate; soil occupation; root compartment; and bacterial genera. The intention is to bring light into the P biosolubilization process, helping to make it more efficient, more accessible and contributing to a more sustainable agriculture.

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S5-L-02
Arbuscular mycorrhizal symbioses as biotechnological tools to increase plant tolerance to climate change

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At present, we are witnessing accelerated changes in the evolution of the climate. These changes lead to an increase in temperature, alterations in precipitation patterns and changes in atmospheric composition, among others. This poses a significant risk to plant survival and involve the need to improve acclimatization processes. Arbuscular mycorrhizal (AM) fungi played a fundamental role in the terrestrialization of plants in early Palaeozoic, helping them to cope with the challenges of moving from an aqueous medium to land (desiccation, heat, UV radiation damage, nutrition absorption). The almost universal presence of AM fungi in terrestrial environments together with their ability to increase plant tolerance and resilience to multiple stressors, both biotic and abiotic, points to AMF as biotechnological tools to facilitate the plant physiological adjustment to the new climatic conditions. In this presentation we will analyze the main effects produced by the establishment of the AM symbiosis in the physiology of the plant in relation to the stresses most linked to climate change (drought and salinity, increased temperatures and pathogen and pest attack) and what we know about the mechanisms involved in these effects. We will discuss about the main effects of climate change on AM formation and functioning and summarize our preliminary results on the mechanisms involved in the higher tolerance of tomato plants in symbiosis with different AM fungi to high temperatures.

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S5-O-01
Host susceptibility factor MLO facilitates symbiosis with beneficial microbes

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Understanding how plants balance the ability to both resist pathogens and accommodate symbionts has direct implications for fundamental plant biology and the optimal use of crop plants in agriculture.
Host susceptibility genes enable the colonization of plants by harmful pathogens. Mildew resistance locus O (MLO) is a host susceptibility factor, first identified in barley, which confers infection by powdery mildew (*Blumeria graminis*), a biotrophic fungal leaf pathogen. In loss-of-function *mlo* mutants, fungal development is restricted at host cell entry. Thus, *mlo* mutants provide robust and durable immunity.
We considered, why plants have susceptibility factors. Since MLO's function in facilitating powdery mildew infection is disadvantageous to the host, it follows that it must also fulfil some other beneficial role that explains its conservation throughout evolutionary history. We reasoned that MLO may have a role in supporting an ancient beneficial plant-microbe interaction - the arbuscular mycorrhizal symbiosis.
Based on the increased expression of MLO in roots colonized by arbuscular mycorrhizal fungi and its presence in a clade of the MLO family that is specific to mycorrhizal-host species, we investigated the role of MLO in arbuscular mycorrhizal interactions. Using mutants from crops, barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), and model species, *Medicago truncatula*, we demonstrate a role for MLO in colonization by the arbuscular mycorrhizal fungus *Rhizophagus irregularis.*
Early mycorrhizal colonization was reduced in *mlo* mutants of barley, wheat, and *M. truncatula,* and this was accompanied by a pronounced decrease in the expression of many of the key genes required for intracellular accommodation of arbuscular mycorrhizal fungi. Our findings show that clade IV MLOs are involved in the establishment of symbiotic associations with beneficial fungi, a role that has been hijacked by powdery mildew.
The colonization of plants by mycorrhizal fungi and by symbiotic nitrogen-fixing bacteria share many conserved genes and a common signalling pathway. Ongoing work is investigating whether MLO also has a role in promoting colonization by nitrogen-fixing bacteria.

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S5-O-02
Graphene oxide influence in soil bacteria is dose dependent and changes at osmotic stress: growth variation, oxidative damage, antioxidant response and plant growth promotion traits of a *Rhizobium* strain

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Climate change events, such as drought, are increasing and soil bacteria can be severely affected. Moreover, the accumulation of emerging pollutants are expected to rapidly increase, and their impact on soil organisms, their interactions, and the services they provide is poorly known. The use of graphene oxide (GO) has been increasing due to their enormous potential for application in several areas and it is expected that concentration in soil will increase in the future, potentially causing disturbances in soil microorganisms not yet identified. Here we show the effects that GO nanosheets can cause on soil bacteria, in particular those that promote plant growth, in control and 10% polyethylene glycol (PEG) conditions. Low concentrations of GO nanosheets did not affect the growth of Rhizobium strain E20-8, but under osmotic stress (PEG) GO decreased bacterial growth even at lower concentrations. GO caused oxidative stress, with antioxidant mechanisms being induced to restrain damage, effectively at lower concentrations, but less effectively at higher concentrations, and oxidative damage overcame. Under osmotic stress, alginate and glycine betaine osmoregulated the bacteria. Simultaneous exposure to PEG and GO induced oxidative damage. Plant growth promotion traits (indole acetic acid and siderophores production) were increased by osmotic stress and GO did not disturb these abilities. In a context of climate change our findings might be relevant as they can form the premises for the implementation of crop production methodologies adapted to the new prevailing conditions, that include the presence of nanoparticles in the soil and more frequent and severe drought.

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S5-O-03
Enhanced legume growth and adaptation to degraded estuarine soils using *Pseudomonas* nodule endophytes

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The joint estuary of Tinto and Odiel rivers (SW Spain) is one of the most degraded and polluted areas in the world and its recovery is mandatory. Legumes and their associated bacteria are recommended sustainable tools to fight against soils degradation and loss of fertility due to their known positive impacts on soils. In this work, 33 bacteria were isolated from inside nodules of *Medicago* spp. naturally growing in the estuary of the Tinto and Odiel Rivers. They were genetically and phenotypically characterized by determining plant growth promoting properties, enzymatic activities and tolerance towards As, Cd, Cu and Zn. The best rhizobia and non-rhizobia based on the studied characteristics were selected. Strains identified as *Pseudomonas* sp. N4, *Pseudomonas* sp. N8, *Ensifer* sp. N10 and *Ensifer sp.* N12, were used to inoculate *Medicago sativa* plants. The effects of individual or combined inoculation on seed germination and plant growth and nodulation were studied, both on plates and pots containing poor-nutrient soils from the contaminated estuary. Co-inoculation with *Ensifer* and *Pseudomonas* increased plant biomass and nodules number compared to single inoculation with rhizobia, ameliorating the physiological state of the plants and helping to regulate plant stress mechanisms. The best results were observed in plants inoculated with the *consortium* containing the four strains. In addition, inoculation with *Ensifer-Pseudomonas* couples increased metals accumulation in *M. sativa* roots, without significant differences in metal accumulation in shoots. These results suggest that plant growth promoting nodule endophytes (PGPNE) are useful biotools to promote legume adaptation, growth and phytostabilization potential in nutrient-poor and/or metals contaminated estuarine soils.

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S5-P-01
Coffee Agroforestry: effects of shade trees on the rhizosphere of *Coffea arabica* established in the rainforest of the Gorongosa Mountain (Mozambique)

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The Gorongosa National Park (GNP) in Mozambique is a renowned world heritage wildlife and biodiversity area in Mozambique. After years of civil war, GNP is a remarkable story of wildlife restoration involving the surrounding communities to support conservation [1,2].To create employment for smallholder farmers while promoting rainforest reforestation, an agroforestry system of *Coffea arabica* with native shade trees has been established in the Gorongosa Mountain involving sustainable farming practices. To date, however, studies showing the outcomes of shade tree integration, and the potential impacts on the rhizosphere of adjacent coffee trees are still missing. This study, based on high-throughput sequencing, explores the effects of shade trees on the diversity, structure, and composition of *C. arabica* rhizosphere communities (Bacteria, Fungi, and Archaea) grown at different elevations (600m, 800m, and 900m) and under different levels of canopy shading (no shadow, 50% and 100% of shadow from native trees) [3]. Alpha-diversity (observed operational taxonomic units, Chao 1 and Shannon index) was significantly different for the three shade levels. For Bacteria, coffee rhizosphere collected at 600m showed a higher diversity under 100% of shade trees, while at 800m the diversity was higher without shade, and at 900m under 50% of shade. For Archaea, at 600m, the highest diversity was observed under 100% of shade, while in the remaining altitudes it was higher under 50%. For Fungi, the highest diversity at 600 and 800m was observed under 50% shade, and at 900m in the rhizosphere without shade. The most abundant genera were Chthoniobacter for Bacteria, Nitrososphaera for Archaea and Linnemannia for Fungi. This study demonstrates the benefits of shade trees in this agroforestry system and emphasizes the rhizosphere as a key link in indirect impacts of shade trees on the health and productivity of *C. arabica* in diverse systems.

Keywords: Agroforestry system, Gorongosa National Park, *Coffea arabica*, Shade trees, Rhizosphere Microbiome.

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S5-P-02
Interactions between *Tuber melanosporum*, aromatic plants and associated arbuscular mycorrhizal fungi in intercropping designs

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Intercropping black truffle (*Tuber melanosporum* Vitt.) plantations is a promising way to increase ecosystem services provided by truffle culture i.e., alternative productivity before truffle fruiting, increase population of pollinators and soil health status. However, truffle brûlés, the areas with scarce vegetation due to the allelopathic effects of truffle mycelium in the soil, could limit intercropping possibilities by reducing plant growth and associated soil microbiota (Streiblová et al. 2012). Among compatible crops, aromatic plant species are a suitable alternative for Mediterranean truffle plantations since they are well adapted to the same ecological conditions as truffle oaks. We have designed a mesocosm experiment to evaluate the interactions between mycorrhizal evergreen oaks and three species of aromatic plants: *Lavandula officinalis* L., *Thymus vulgaris* L. and *Salvia officinalis* L., all of them forming arbuscular mycorrhizal (AM) symbiosis. Native AM fungi (AMF) were isolated from weeds growing in productive truffle brûlées and inoculated on the target plantlets. One-year-old truffle oaks from a commercial nursery were co-cultivated in sterilized soil with either, mycorrhizal or non-mycorrhizal aromatic plants (one oak per 7 L container surrounded by three aromatic plants of the same species). Two control treatments included oaks and mycorrhized aromatic plants growing alone. After 12 months in a greenhouse with regular watering, growth parameters and mycorrhizal colonisation (both from *T. melanosporum* and AMF) of plants were measured in all treatments. Soil mycelium from *T. melanosporum* was quantified by Taqman® qPCR according to Parladé et al. (2013). AMF soil biomass was estimated by comparative C_T quantification ($\Delta\Delta C_T$) using generic primers AM1 and AMG1F for Glomeromycota (Bodenhausen et al. 2021) and the internal transcribed spacer (ITS) of rDNA as endogenous control. Aromatic plants inoculated with native AMF fungi showed, in general, higher shoot biomass than non-inoculated ones. The 'brûlé' effect (presence of *T. melanosporum*) caused a growth reduction of all the non-inoculated aromatic plants. However, plants colonized by native AMF showed a higher biomass and only *L. officinalis* was negatively affected by the presence of *T. melanosporum*. The presence of aromatic plants significantly decreased both mycorrhizas and soil mycelium of *T. melanosporum* compared to control oaks growing alone. This reduction was significantly higher when aromatic plants were colonised by AMF. Soil mycelium of *T. melanosporum* was negatively correlated with relative AMF soil biomass. Also, mycorrhizal colonisation and soil biomass were positively correlated for both, *T. melanosporum* (in oaks) and AMF (in aromatic plants). These results show the strong competition of AMF and *T. melanosporum* in the brûlé and the potential protection of intercropping aromatic plants from allelopathic effects in truffle plantations.

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S5-P-03
Differences in P uptake mediated by solubilising and non-solubilising phosphate PGPR in wheat plants

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Phosphate solubilisation is a recognised direct mechanism of growth promotion (Glick, 2012). Therefore, solubilising phosphate rhizobacteria able to provide solubilised P to plants act as a source of phosphorus for plant nutrition (Etesami & Maheshwari, 2018). Likewise other PGPR could be involved in helping plants to assimilate phosphorus. Many of the research is focused in the demonstration of qualitative and quantitative ability of PGPR to solubilise P, but the interaction with plants and P dynamics have been less studied. Thus, the present work attempts to analyse this behaviour.

Wheat plants were established in growth chamber and grown under different phosphorus fertilisation conditions with PGPR bacteria belonging to species of *Bacillus*, *Pseudomonas* and *Azotobacter*, with the aim of evaluate the P assimilation and solubilisation. The assay was kept for 5 weeks. Fresh and dry weight, and P plant content were determined at the end of the assay. Relative expression of Phosphate Transporters (PTs) genes PT1.2 and PT4 responsible for the direct inorganic P (Pi) uptake pathway (Duan et al., 2015) in roots, were analysed in order to understand the P metabolism when inoculated plants with bacteria are exposed to P deficiencies.

Results showed an increase in P content in aerial biomass of wheat plants inoculated with PGPR and fertilised with half of P dose. These results indicate an improvement in the P uptake mediated by the PGPR action. According to the analysis of the gene expression of PT1.2 and PT4, the down regulation of PT4 in plants inoculated with strains of *Azotobacter salinestris*, *Pseudomonas koreensis*, *Bacillus siamensis* and *Pseudomonas brasicacearum* subsp. *neoaurantiaca*, explains the uptake of P, while the down regulation of PT1.2, explains the P uptake in plants inoculated with *Azotobacter chroococcum* and *A. salinestris*. On the other hand, there is a clear decrease in P uptake in plants inoculated with P solubilising bacteria and fertilised with insoluble P: $Ca_3(PO_4)_2$. The upregulation of Pi-starvation genes PT1.2 and PT4 explains the lack of P uptake in the plant. Although the P solubilising bacteria are able to solubilise P, it is hypothesized that at the short time the bacteria use this P for its own growth and once acquire its maximum growth, pass the P to the plant, making it available for the plant at medium or long term.

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S5-P-04
Plant growth-promoting traits of maize bacteria isolated from different water regimes

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Alterations in precipitation and soil moisture, along with temperature increase in the past decade, are threatening agricultural productivity in the Iberian Peninsula and rest of the world. Due to the selection of crops that produce high yields, but also require high consumption of water, drought is considered a significant abiotic stress in agriculture. Thus, there is urgent need for sustainable crop production more resilient to water deficit and at the same time economically viable. To accomplish this goal new agricultural methodologies must be explored. Maize is a principal source of food for millions of people worldwide. Its high nutritional profile and non-allergenic properties make maize an important world cereal. The high susceptibility of maize to drought implies a high dependence on water for maize growth. The decrease of available water for irrigation could decrease the areas suitable for maize production with consequences for assuring food supply in the future for millions of people. Consequently, promoting maize resilience to drought is a significant step towards food security and adaptation to climate change. The use of microorganisms capable of promoting plant growth in drought environments is a potential approach to mitigate crop losses, since soil microbial communities can play an important role in plant growth and tolerance to stress, with a positive impact on crop productivity. Plant growth-promoting bacteria help plants survive in stressed environments by promoting faster germination and development. Here we describe the isolation of surface and endophytic bacteria from maize plants roots growing in INOVMILHO-ANPROMIS (Santarém, Portugal) exposed to three different water regimes (100%, 50% and 0% of irrigation) in two different stages of their life cycle (vegetative and reproductive). Colony forming units in the soil were also quantified in each condition, and the number of bacterial cells decreased with water availability, as expected. Plants biochemical status was also assessed, to understand if the plants were under stress, and to serve as a basal data for further inoculation studies with specific isolates. Isolates were typed using BOX-PCR to screen for isolates with unique fingerprints. This allowed us to obtain 405 strains. These strains were screened for the ability to tolerate osmotic stress using 15% of polyethylene glycol 6000. The isolates displaying the higher ability to tolerate osmotic stress were tested for bacterial plant-growth promoting traits, namely the ability to produce siderophores, indol-3-acetic acid and phosphate solubilization, in the presence and absence of osmotic stress. The isolation of bacteria from different water availabilities can help to select bacteria effective in the improvement of crop resilience to drought-affected areas, helping in the management of climate change impacts by enhancing plant resilience to drought.

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S5-P-05
Biotechnological potential of *Pantoea* wheat seed endophytes

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Global food demands and the impact of climate change in agriculture call for affordable bio-based products to improve plant productivity while preserving natural resources. Plant growth-promoting (PGP) microorganisms are optimal candidates to improve plant yields under sustainable agricultural conditions. The ability of certain PGP-bacteria of providing nutrients or increasing plant tolerance to stress is well known. Most studies so far have focused on the N-fixing association of *Rhizobium* with legumes. However, the role of other bacteria that establish close interactions with plants, like those dwelling seeds, remains yet under-explored.

In this work, we evaluate the composition and role of endophytic bacterial communities from wheat, a daily-consumed cereal which production has increased over the last years. For that, commercial (*Triticum aestivum*) and ancestral (*T. spelta*) wheat seeds harvested from different fields were submitted to cultivation-dependent and -independent analysis. 16S metataxonomy revealed that seed-borne wheat microbiota assemblages are conserved and dominated by bacteria belonging to the *Pantoea* genus. Furthermore, endophytic bacterial isolates from wheat seed samples were screened *in vitro* for PGP-abilities like mobilization of P K, or synthesis of indole acetic acid (IAA). Selected strains were further tested in growth chamber, greenhouse and field experiments with broadacre and horticulture crop references. Several *Pantoea* isolates show positive effects on seed germination, root and plant development, even under hydric stress. Our results highlight relevance of seed-borne bacteria as plant biostimulants.

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S5-P-06
The nodule endophytic acetic acid bacterium *Endobacter medicaginis* promotes the growth of alfalfa in acidic soils

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Alfalfa (*Medicago sativa* L.) is a forage plant cultivated worldwide for thousands of years and whose production can be maintained during several years after the sowing (Patra and Paul, 2021). The global alfalfa market is expected to grow with a CAGR (compounded annual growth rate) of around 7% (https://www.fortunebusinessinsights.com/alfalfa-pellets-market-103597). However, the cultivation of this legume is limited in some stressed soils, such as acidic ones (Ramírez-Bahena et al. 2015) and bacterial biofertilization is desirable to increase the production of this legume in these soils. In the present work we analysed the effect on alfalfa growth of the strain M1MS02, which is the type strain of *Endobacter medicaginis*, an acetic acid bacterium isolated from a nodule of alfalfa growing in an acidic soil. Acetic acid bacteria have different *in vitro* mechanisms involved in plant growth promotion (Pedraza 2016). The genome mining showed that strain M1MS02 harbours different genes involved in plant growth promotion mechanisms such as phosphate solubilisation or indole acetic acid production, also confirmed by in vitro analyses. The results of inoculation experiments of the strain M1MS02 carried out in hydroponic, microcosms and field conditions showed that this strain was able to promote the growth of this legume. Therefore, *E. medicaginis* M1MS02 can be considered as a good candidate for alfalfa biofertilization in acidic soils.

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S5-P-07
Arbuscular mycorrhizas modulate the physiological and transcriptomic responses of tomato plants to heat stress

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Climate change, entailing shifts in temperature (T), precipitation and atmospheric composition among other factors, represents a moving target for plant adaptation. The widespread distribution of arbuscular mycorrhizal (AM) fungi and their ability to increase plant stress resistance has led to the suggestion that they can be key drivers in increasing plant resilience to climate change. Changes in atmospheric conditions and global and regional climate affect AM functioning, yet the potential role of AM symbioses in mediating plant responses to global change and the underlying mechanisms remain unexplored. This study was aimed at studying the physiological and transcriptomic responses of tomato plants inoculated with *Rhizophagus irregularis* DAOM 181602 or *Claroideoglomus etunicatum* (isolated from a stressful ecosystem in Spain) to high T (34 ºC). Heat stress had a negative effect on plant biomass in all treatments, but to a lower extent in mycorrhizal plants. High T inhibited root colonization by *R. irregularis* but not by *C. etunicatum*. Both AM fungal species mitigated the impact of heat stress on the tomato ionome. Root transcriptome profiles showed significant differential expression of 3909 transcripts under heat stress in non-mycorrhizal roots and of 3363 and 3575 genes in *R. irregularis*- and *C. etunicatum*-colonized roots, respectively. Gene ontology (GO) enrichment analysis revealed that transcripts involved in processes such as “drug metabolism”, “hydrogen peroxide metabolism”, “reactive oxygen species metabolism” and “cofactor catabolism” were influenced by heat stress in all situations. Specific GO processes enriched in non-mycorrhizal roots were related to “transmembrane transport activity”, which could explain the impact of heat stress on their ionome and its mitigation by the AM symbiosis. Specific changes induced in the transcriptome profiles of roots of the different treatments will be further discussed.

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S5-P-08
Evaluating the effect of various bacterial consortia isolated from arid wild legumes on heat stress tolerance of *Pisum sativum*

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High-temperature stress affects the growth and developmental process of cool-season grain legumes. We hypothesized that endophytic bacteria associated with arid plants could be a potential resource to ensure the tolerance of cold-season legumes to high temperature stress events. To test our hypothesis, *Phyllobacterium salinitolerans* (PH), *Starkeya* sp. (ST) and *Pseudomonas turukhanskensis* (PS) endophytes of different spontaneous legumes localised in Tunisian arid regions were selected to evaluate their potential in improving *Pisum sativum* growth and pea-rhizobia symbiosis under a heat stress event. Three consortia (containing different combinations of endophytes) were used along with the pea microsymbiont *Rhizobium leguminosarum* 128C53 (WT) or with its Δ*acdS mutant* derivative (MT) (Ma et *al*., 2003). Uninoculated plants without or with nitrogen supplement were used as negative (NC) or positive controls (PC), respectively. The heat stress event was applied 2 weeks after sowing for a period of 2 weeks with consecutive cycles of 30-35°C/16h and 20°C/8h. Interestingly, the shoot dry weight (SDW) of all plants co-inoculated with WT and any of the consortia containing PH increased significantly compared to that of plants inoculated with WT alone. A similar effect was observed on the root dry weight (RDW) in the treatments WT+ST+PH and WT+PS+PH. On the other hand, the best results either in terms of SDW or RDW with the mutant strain was the treatment that included all endophytes (MT+ST+PS+PH), even overcoming all treatments inoculated with WT and equalling the PC. As expected, plants inoculated with the MT had a lower number of nodules (NN) compared to plants inoculated with WT, except for MT+ST+PS+PH with similar NN. A significant increase in the NN was observed in plants co-inoculated with WT+ST+PH and WT+PS+PH compared to those in WT. The highest total chlorophyll content was in WT+ST+PS, which was significantly different from all other treatments while no differences were observed in phenolic compounds content among the inoculated treatments. Overall, our results suggest that endophytic isolates from arid leguminous plants are good candidates for increasing the resilience of plants not adapted to heat stress.

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S5-P-09
Optimizing legume nodulation in situations of environmental stress: inoculants with multiresistant endophytes

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Climate change is one of the biggest problems today, and it has caused different types of abiotic stress in soils that were suitable for crops. The abuse of agrochemicals, heavy metals and other pollutants increasingly affects agricultural soils. This contributes to the loss of nutrients and beneficial microorganisms, which is why solutions are needed that can improve plant growth even in these environmental conditions. Plant growth promoting bacteria (PGPB) can be used as inoculants to increase the nodulation of leguminous plants and are an excellent option for the recovery of degraded soils through the biological fixation of N_2 (Navarro-Torre et al., 2020 and Flores-Duarte et al., 2022). Endophytic bacteria (plant growth promoting endophytes: PGPE) isolated from nodules of the rhizosphere of legumes (*Medicago* spp.) from the Marismas del Rio Odiel, Huelva, Spain, grown in soils affected by heavy metals, have been used in this work. The selected bacteria belong to the genera *Ensifer* and *Pseudomonas*. Tolerance to heavy metals, salinity, pH and high temperatures were determined. At the same time, their plant growth promoting properties, PGP (auxin production, N_2 fixation, ACC deaminase activity, etc.) were verified. On the other hand, lytic enzymes such as cellulase, pectinase, etc. were investigated. Finally, the following bacteria were used: *Ensifer* sp. N10 and N12 as rhizobia and the endophytes of the nodule *Pseudomonas* sp. N4 and N8. These bacteria have been used to make inoculants to improve germination, biomass and nodulation of alfalfa (*Medicago sativa*) under stress conditions. *In vitro* nodulation studies were carried out with *M. sativa* in the absence and presence of arsenic (30 µM As), germination without and with a mixture of metals (7.5 µM As, Cd, Cu and Zn) as well as in the greenhouse nutrient-poor soils, contaminated with metals and salt (60 mM), with the following treatments: control non-inoculation (C-), inoculation with the rhizobium (N10) and inoculation with a consortium of both *Pseudomonas* and the two rhizobia (CSN) . The results obtained showed an increase in germination in the absence and presence of metals with the CSN *consortium*, of **13**% and **34**%, respectively, compared to the non-inoculation (C-). Similarly, inoculation with the (CSN) consortium showed **73**% more nodules in the absence, and a **91**% increase in the presence of As, compared to *rhizobium* N10. Regarding the results of nodulation under greenhouse conditions with the consortium (CSN), the number of nodules increased in nutrient-poor soils (**104**%), with metals (**60**%) and soils with salt (**72**%), compared to with N10. The results of biomass, root length and shoot followed this pattern: **CSN>N10>C**-. These results suggest that the inoculation of legumes with the consortium (CSN) composed of multiresistant rhizobia and PGPE is a useful tool to promote their growth in soils with different types of abiotic stress and, in turn, guarantee the minimum accumulation of metals in the soil. aerial part of the plants, to be able to use them both in the recovery of estuaries and as forage plants.

Keywords: abiotic stress, arsenic, PGPE, heavy metals, high temperature, legumes, multiresistant, nodulation, PGPB, salt.

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S5-P-10
Effect of tree canopy and dolomitic limestone application on soil microorganisms and pasture quality in the *Montado* ecosystem

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The *Montado*, is an agro-silvo-pastoral ecosystem, explored in several extents. Its arboreal stratum is mainly composed by cork oak (*Quercus suber*) and holm oak (*Q. rotundifolia*) while the ground cover is formed by natural biodiverse pastures growing on poor soils and grazed by animals in an extensive regime. One of the major factors affecting the productivity of pastures in the *Montado* system is soil acidity (Serrano et al., 2020). The objective of this work is to evaluate the effect of tree canopy and dolomitic limestone application for soil acidity correction on soil microbial biomass carbon, arbuscular mycorrhiza colonisation rate, pasture floristic diversity and quality in the *Montado* ecosystem. Soil and root samples were collected in an experimental field (4ha) located in Mitra Farm in February 2022.In April we monitored the floristic composition of the pasture and measured its quality through the qualification of crude protein (CP) and fiber (neutral detergent fiber- NDF) content. The results showed that there was a positive and significant effect of the canopy on microbial carbon biomass and mycorrhizal colonization, and also significant differences on mycorrhizal colonization related to soil pH amendments, particularly under the tree canopy. Despite the lack of significant differences in CP and NDF, the average values for both parameters were higher under the canopy. Considering CP it was higher in the amended area, while NDF was lower in the same area. These results allowed us to perceive the effect of organic matter deposition associated to the tree canopy and soil acidity correction as key factors for microbial development (Cardoso & Andreote, 2016; Rodrigues et al., 2015) and pasture floristic diversity and quality in the *Montado* system, providing important information regarding the holistic management of this ecosystem.

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S5-P-11
Response of spore density and root colonization by arbuscular mycorrhizal fungi to the introduction of cover crops in intensive production of maize

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Beneficial microorganisms occur naturally in little-intervened ecosystems, but many practices associated with intensive agriculture result in biodiversity imbalances that disadvantage them relative to other non-beneficial species (Graham, J.H. 2000). In these intensive systems, the occupation of the soil in the autumn-winter period with cover crops based on legumes, grasses, or biodiverse mixtures of both, will increase the enrichment of the soil in beneficial microorganisms, which will be available to interact with the main crop. The adoption of practices that favour mycorrhization can also play a very important role (Schloter *et al*., 2018). Mycorrhiza is a mutualistic relationship between plant roots and fungal hyphae.

This work describes the evaluation of the degree of mycorrhization and spore density, as a soil biological indicator, in an experimental field in Golegã (Portugal), where different cover crops were installed prior to corn (*Zea mays* (L.) cultivation: 1) biodiverse mixture of grasses and legumes, including rhizobia-inoculated clovers; 2) *Lolium multiflorum* (Lam.) (annual ryegrass), a mycotrophic grass that favours soil enrichment in endemic endomycorrhizal fungi; and 3) *Raphanus sativus* (L.) (forage turnip), a biofumigant species which its incorporation into soil contributes to the elimination of phytopathogens. A control plot was maintained without any cover crop. Samples of roots from corn were collected and the degree of endomycorrhization was evaluated.

The obtained results indicate that the introduction of cover crops, in particular the biodiverse mixture and annual ryegrass, increased the mycorrhization frequency in corn roots and the density of spores in the soil.

The arbuscular mycorrhiza fungal propagules can be developed in the soil by mycotrophic plants and kept intact at the seeding of the corn crop by adopting appropriate tillage techniques. This work points out the importance of cover crops for the enrichment of these systems in endomycorrhizal fungi.

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S5-P-12
Arbuscular mycorrhizal fungi as indicators of soil conservation status in dryland degraded area

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This research is part of an innovative pilot project for habitat restoration on the Famara summits in Lanzarote, Canary Islands, which aims to be a starting point for the recovery of the native vegetation of this arid area and to halt the desertification process suffered by the whole island. The project is led by the Department of Ecological Transition, Fight against Climate Change and Territorial Planning of the Government of the Canary Islands, and financed with FEDER resources. It has been suggested that a common reason for the failure of many restoration attempts is to omit the necessary and direct relationship between the root system and the diverse community of microorganism (3). For this reason, we propose as a first step in the approach to restoration, the study of a particular group of microorganisms: the arbuscular mycorrhizal fungi (AMF). These organisms are ubiquitous soil-borne microbial fungi that play a critical role in plant nutrition which has become an integral component of plant ecology in natural ecosystems. Especially AMF play key roles in semiarid ecosystems, improving the function and adaptation of plant communities to these stressed environments (2).

The present study investigates the native AMF population in damaged soils, as indicator of degradation. In addition, the mycorrhizal potential of the different soils has been determined in order to select the most optimal areas to restore and reproduce mycorrhizal inoculum. Simultaneously, the most suitable trap culture was assessed. Finally, the relationship between arbuscular mycorrhizal fungi population and soil physico-chemical parameters has been evaluated. Firstly, in order to achieve these objectives, different states of degradation of the soil system have been determined. The classification criteria applied were the presence of soil horizons and the presence or absence of vegetation and its characteristics. A total of seven locations representing the different stages of degradation have been identified. From each location, 7 composite samples were obtained and processed. The first step in assessing the AMF population has been to count spores per gram of soil according to the wet sieving protocol (1). Then, the mycorrhizal potential has been determined by different trap cultures, once with mycotrophic allochthonous plants and once with native leguminous and mycotrophic plants. After three months, the percentage of root mycorrhization and the number of spores were evaluated. As expected, the number of spores obtained has been significantly higher in the vegetation islands. On the other hand, the worse results have been obtained where the gully erosion occurs and in cliff areas, where there is hardly any soil system but where some isolated endemic botanical species are found. Based on these results, it can be concluded that this indicator appears to be sensitive to the soil degradation. Furthermore, the best-preserved AMF population is directly linked to higher organic matter and less phosphorus and magnesium available in soil. When we evaluated the mycorrhizal potential, the results have shown a greater ecological potential in soils with high plant diversity, such as vegetation islands, and in soils with annual and spontaneous vegetation Soils with volcanic mineral mulch called "picón" also had better AMF populations. According to these findings, these areas will be native mycorrhizal reservoir and susceptible areas for restoration. Finally, in relation to the best trap culture, native leguminous plants shown to reproduce the AMF better.

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XVIII SEFIN OEIRAS '22

BeMiPlant

Beneficial Plant-Microbe Interactions

SESSION 6

Genetics and "Omics" of Plants and Associated Microbes

S6-L-01
Is ethylene required for an active nitrogen fixation and nodule development in *Medicago truncatula*?

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The plant hormone ethylene is a negative regulator of the establishment of most legume-rhizobium symbiotic interactions. The identification of the *Medicago truncatula* mutant *sickle* (*skl*, Penmetsa and Cook, 1997) has allowed understanding some of the molecular mechanisms controlling the early stages of this interaction. For instance, it is now known that ethylene inhibits root hair deformation, calcium spiking, and infection thread initiation and growth (Oldroyd et al., 2001; Penmetsa and Cook, 1997). However, the role of ethylene at later symbiotic stages has barely been analyzed.

In the current work we have tested the hypothesis that ethylene signalling positively regulates nodule development and symbiotic nitrogen fixation in *Medicago truncatula*. To test it, we have compared the growth characteristics and symbiotic performance of the ethylene insensitive mutant *skl* and the wild type *M. truncatula* A17 genotype. Physiological characterization of the plants shows that both genotypes present similar plant biomass. *skl* shows an hypernodulating phenotype, with smaller, rounder nodules, but with a bacteroid colonization comparable to this of the wild type. We tested its ability to fix N_2 using several complementary approaches: measuring apparent nitrogenase activity, using a *Sinorhizobium meliloti* strain carrying the marker for nitrogenase expression p*NifH:GUS* and determining the total N content in the plants. Results show that, when grown exclusively under symbiotic conditions, *skl* fixes N_2 at rates comparable with these of A17 plants, expressing nitrogenase in the infected N_2-fixing zones of the nodule and presenting similar N content levels than the wild type genotype. Histological analysis of the nodule structure suggests that *skl* nodules present altered zonation and meristem formation. Additionally, we have observed that the establishment of nodule senescence shows a delay in *skl,* increasing the life span of the mutants. Taken together, our results suggest that ethylene signalling *per se* is not required to establish an efficient N_2-fixing symbiosis, but it may play a role in nodule differentiation and senescence.

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S6-L-02
Structure and function of the α-rhizobia non-coding transcriptome investigated by RNAseq

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Deep sequencing of strand-specific cDNA libraries (RNAseq) has unveiled unexpectedly complex transcriptional outputs from prokaryotic genomes, adding large and heterogeneous inventories of small non-coding transcripts (sRNAs) to the classical translation-related ribosomal (rRNA), transfer (tRNA) and messenger (mRNA) RNA species (Hör *et al*. 2018). Most sRNAs regulate extensive post-transcriptional networks underlying virtually any adaptive trait in bacteria. Therefore, it is increasingly evident that no microbial process can be understood in its full dimension without assessing regulation of gene expression by RNA (riboregulation). Yet inconceivably, sRNAs are systematically overlooked in the annotation of bacterial genomes and riboregulation remains largely unexplored in most bacterial species.

We have used cutting-edge RNAseq protocols such as Differential RNAseq (dRNAseq), Cappable-seq or Term-seq to determine transcripts boundaries genome-wide in three α-rhizobial species, *Sinorhizobium meliloti, S. fredii and Rhizobium tropici*, as the model symbionts of the agronomically relevant legumes, alfalfa, soybean, and common bean, respectively. These approaches enabled the accurate annotation of protein-coding sequences, untranslated mRNA regions, and sRNAs in all three genomes. The latter were further catalogued as sense, antisense or *trans* according to the location of their *loci* with respect to the annotated ORFs. Classical RNAseq was then used to explore the symbiotic-dependent alterations of the non-coding transcriptomes, which identified differentially expressed sRNAs with putative symbiotic functions.

The so-called *trans*-sRNAs are expressed from transcriptionally regulated promoters within intergenic regions and rely on protein-assisted short and discontinuous base-pairing to regulate translation and stability of multiple *trans*-encoded mRNA targets. Accordingly, we also used RNAseq to profile the RNA ligands of the major bacterial RNA matchmaker Hfq, and the mRNA interactomes of well-characterized *trans*-sRNAs in *S. meliloti* (Torres-Quesada *et al.*, 2014; García-Tomsig *et al.*, 2022). In the communication, we will provide insights into these RNAseq-based approaches to identify and unravel the function of sRNAs in α-rhizobia.

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S6-O-01
Engineered Calmodulin modulates nuclear calcium oscillation and enhances legume root nodule symbiosis

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Oscillations in intracellular calcium concentration play an essential role in the regulation of multiple cellular processes. In plants capable of root endosymbiosis with nitrogen-fixing bacteria (rhizobia) and/or arbuscular mycorrhizal fungi (AMF), nuclear-localized calcium oscillations are essential for the establishment of these interactions.

The entry of rhizobia and AMF into legume roots is initiated by the recognition of the endosymbiont. Host plants have plasma membrane receptor-like kinases that recognize rhizobial and fungal elicitors. This recognition triggers the activation of calcium oscillations in root epidermal nuclei to initiate the endosymbiosis program. In *Medicago truncatula* model legume, the fluctuations in nucleoplasmic calcium concentrations are generated by ion channels located at the nuclear envelope, including the CYCLIC NUCLEOTIDE GATED CHANNELS 15 (CNGC15s) (Charpentier et al., 2016). However, how the CNGC15s are regulated in planta to sustain a calcium oscillatory mechanism remains unknown.

In this study, we demonstrate that CNGC15s are regulated by the calcium-bound form of the Calmodulin 2 (holo-CaM2) in planta, which shapes the oscillatory pattern of nucleoplasmic calcium concentration by providing negative feedback on CNGC15s to cause its closure. By engineering CaM2 to generate $CaM2^{R91A}$ mutant, which specifically increased holo-CaM2 binding affinity to CNGC15, we accelerated closure of CNGC15s and increased the calcium oscillation frequency. We further show that accelerating the calcium oscillation frequency was sufficient to accelerate the early endosymbiosis signaling and that the expression of $CaM2^{R91A}$ resulted in an enhanced root nodule symbiosis but not enhanced AMF colonization. Our data reveal differential regulation of rhizobia and AMF endosymbioses and suggest that modulating calcium signaling can be used as a strategy to positively impact symbiosis with nitrogen-fixing bacteria.

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S6-O-02
Genomic characteristics and comparative genomics analyses of non-rhizobial endophytic bacteria isolated from legumes

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In addition to typical nitrogen-fixing endosymbionts, legumes harbour other endophytic bacteria within their tissues that may also contribute to plant growth and health (Hardoim *et al. 2015)*. Our previous works revealed that endophytic bacteria isolated from legumes not only have plant growth-promoting traits but are also well adapted to common constraints present in soils of the Mediterranean region (Brígido *et al.* 2019ab). In this work, we intend to further characterize these endophytic bacteria through genomic and comparative genomic analyses to potentiate their applications in agriculture, providing opportunities for sustainable plant health and food security. Twelve endophytic bacterial isolates were selected based on their potential for plant growth promotion and/or biocontrol of phytopathogens. Based on the 16S rRNA gene sequence analysis, the isolates were assigned to the genera *Pseudomonas*, *Kosakonia*, *Stenotrophomonas*, *Serratia*, *Bacillus* and *Agrobacterium*. Nevertheless, *in silico* DNA-DNA hybridization analyses revealed that 3 strains represent novel species distinct from their closest relatives. Their genome sizes ranged from 4.4 M to 7.1 M with a GC content varying from 35.41 to 66.4%. Orthologous gene clusters analysis revealed 9346 clusters and 345 single-copy gene clusters, albeit only 499 gene clusters (comprising 6110 proteins) were shared among all strains. Whole genome sequence analysis revealed genes potentially associated with attachment and plant colonization, growth promotion and stress protection as well as antifungal activity. In detail, sets of genes for twitching motility, chemotaxis, flagella biosynthesis, and ability to form biofilms (which are related with host plant colonization) were found in their genomes. Presence of genes associated to nitrogen fixation, auxin biosynthesis, siderophore production or phosphorous assimilation reveals their potential as plant growth promoters. Furthermore, genes required for biosynthesis of pyoluteorin, 2,4-diacetylphloroglucinol and pyrrolnitrin underline bacterial biocontrol potential against phytopathogens. Genes related to the production of different molecules and enzymes mediating stress tolerance suggest their ability to rapidly adapt to stressful conditions. Overall, our data provide a better understanding of these endophytic bacteria abilities and further comparative genomic analysis provided insight into the genomic basis of their endophytic lifestyle, plant growth promotion and antifungal activity.

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S6-O-03
Salt stress tolerance in *Casuarina glauca*: insights from the branchlets transcriptome

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Climate change is unequivocally driving major environmental struggles. Together with the accelerated rate of population growth, these changes are imposing a substantial loss of biodiversity and arable land. In this context, the use of pioneer trees has been pointed as a powerful tool to restore degraded lands. Among them, actinorhizal trees – a group of perennial dicotyledonous angiosperms able to establish root-nodule symbiosis with N_2-fixing *Frankia* bacteria - constitute important elements in plant communities worldwide and have been successfully used in land reclamation. In the present work, we have analyzed the transcriptome of the photosynthetic organs of *Casuarina glauca* (branchlets), to unravel the molecular mechanisms underlying stress tolerance. For that, *C. glauca* plants supplied either with chemical nitrogen (KNO_3^+) or nodulated by *Frankia* (NOD^+) were exposed to a gradient of salt concentrations (200, 400, and 600 mM NaCl) and RNA-Seq was performed using an Illumina Platform. An average of ca. 25 million clean reads was obtained for each group of plants, corresponding to 86,202 unigenes with a N50 size of 2,792 bp and 41% GC content. The patterns of differentially expressed genes (DEGs), clearly separate two groups, (i) control and 200 mM NaCl-treated plants, and (ii) 400 and 600 mM NaCl-treated plants. On the other hand, although the number of total transcripts was relatively high in both plant groups, the percentage of significant DEGs was very low, ranging from 6 (200 mM NaCl/NOD^+) to 314 (600 mM NaCl/ KNO_3^+), mostly involving downregulation. The up-regulated genes were mostly related to regulatory processes, reinforcing the hypothesis that some ecotypes of *C. glauca* have a strong stress-responsive system with an extensive set of constitutive defense mechanisms, complemented by a tight mechanism of transcriptional and post-transcriptional regulation. The results highlight the complexity of the molecular interactions leading to salinity tolerance in this species which is probably linked to long-term ecological adaptation and in some cases independent of symbiotic *Frankia.*

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S6-O-04
Engineering membrane vesicles for fine-tuned modulation of rhizobia species interactions for enhanced nodulation and plant growth

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Membrane encased structures serve multiple biological functions in bacteria, including the formation of intracellular compartments and extracellular vesicles, which are essential for bacterial-bacterial communication and bacterial-host interactions[1]. They also hold potential for biotechnological applications, especially in vaccine production, drug delivery, and valuable chemical production. Despite the tremendous advances in elucidating eukaryotic vesicle formation —endocytosis and exocytosis—and identifying key players, the proteins participating in this process in bacteria remain elusive[2].

Although a wide variety of bacterial processes can initiate vesicle formation, little information is available on the systems that coordinate the bacterial membrane's restructuring. My research group, in cooperation with the University of Kent (England), stumbled upon a family of uncharacterized proteins, which are highly conserved across species and actively promote both intra- and extracellular vesicle generation. We systematically produced recombinant proteins of this family in *Escherichia coli* from both Gram positive and negative microbes observing by scanning and transmission electron microscopy striking membrane restructuring.

The production of a protein group led to the formation of intracellular membranous compartments either tubular or globular, whereas the other group boosted extracellular vesicle release. Both structures independently of the cellular compartment are generically termed as membrane vesicles. We investigated the impact of overproducing these proteins in a human pathogen, *Pseudomonas aeruginosa*, whose genome encodes both proteins revealing the same effect. The deletion of both genes in *P. aeruginosa* resulted in a substantial loss of vesicle formation. These results collectively pinpoint these two groups of proteins as active membrane re-shaping effectors, and it is to our knowledge the first report on active vesicle production in bacteria. In this project, I propose to follow up the groundwork laid in *E. coli* and *P. aeruginosa* to harness the full potential of these membrane vesicles for the benefit of rhizobia-plant interactions. Firstly, we will employ the interactomics techniques that are routinely conducted in my lab, which consist of affinity purification coupled with mass spectrometry to identify the protein-protein interactions underlying the biogenesis of these structures in different rhizobia species. Next, we will induce the expression of diverse interaction partners found in the interactomic studies to modulate vesicle formation. Finally, we will tailor the proteic and metabolic cargo of these membrane vesicles encapsulating nodulation factors, nitrogen-fixing enzymes, or phytopathogen inhibitors to improve nodulation, nitrogen fixation, and ultimately plant growth.

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S6-O-05
Relevance of rhizobial T6SS for the interaction with some legumes

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The *Rhizobium*-legume associations are established through specific signals, as those produced by the rhizobia such as Nod factors, exopolysaccharides and secreted proteins called effectors. These effectors are translocated by different secretion systems, and the type VI secretion system (T6SS) is one of them. Genes encoding components of T6SS are present in more than 60% of rhizobia, but the relevance of the system is not well understood in these bacteria. The T6SS has been related mainly to antibacterial activity and virulence against eukaryotes. Our group studies the T6SS of *Rhizobium etli*, *Bradyrhizobium* sp. and *Rhizobium ruizarguesonis*. In the first two species, a positive effect on symbiosis of the T6SS was shown (Salinero-Lanzarote et al., 2019, Tighilt et al., 2021) and in the third one, the T6SS seems to have a negative or neutral effect on symbiosis with peas.

An analysis by mutagenesis of genes encoding potential T6SS-dependent effectors has shown that, in *R. etli*, these effectors would have no relevance to bean symbiosis in contrast to what results from mutation in a structural gene such as *hcp*. The potential effectors would be related to antibacterial functions, as deduced from the presence of conserved motifs, impaired growth when expressed in *E. coli,* and reduced competitiveness in nodulation compared to the wild type strain. In *Bradyrhizobium sp.* a mutation in a methyltransferase encoded by its T6SS has an effect on symbiosis with *Lupinus angustifolius* that could be related to plant recognition of the bacterium (Tighilt et al., 2021).

Regarding the expression of the T6SS systems, we have identified a promoter region (P6) where the orientation of the two main T6SS gene operons diverge. This region is active in symbiosis in *R. etli* and *Bradyrhizobium* sp., whereas it is not active in *R. ruizarguesonis*. P6 activation in free-living conditions has been found only in *R. etli*. The *R. ruizarguesonis* P6 inactivity (deduced from the absence of Hcp immunodetection) may be due to the accumulation of mutations in this region. This is consistent with an incompatibility between T6SS activity and effective nodulation in peas.

These data suggest that, similarly to the presence of the type III secretion systems in rhizobia, the T6SS may have a positive, negative or neutral role in symbiosis depending on the interacting legume and rhizobial species.

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S6-O-06
Characterization of the Glyceraldehyde-3-phosphate dehydrogenase protein in *Rhizobium etli*

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a housekeeping protein widely conserved across all living organisms. Its principal function is the interconversion of glyceraldehyde-3-phosphate and 1,3-bisphosphoglycerate in the glycolytic and gluconeogenic pathways. However, it has also been described as a moonlighting protein involved in different biological processes (Seidler, 2013). In plant beneficial bacteria, such as rhizobia, and in pathogens like *Pseudomonas syringe,* non-conclusive evidence that GAPDH may have a moonlighting function or be exported to the outside of the cell has been reported (Emerich & Krishnan, 2014; Elkhalfi et al., 2014). Results from our laboratory indicate that *Rhizobium etli* GAPDH protein can be secreted to the extracellular culture medium under certain conditions (Lorite et al., in preparation).

With the aim of identifying possible new roles of GAPDH in *R. etli* CFN42, a mutant strain AC1D lacking the *gap* gene was constructed and characterized. We assessed its metabolism in free-living cultures as well as in symbiosis with its host plant, *Phaseolus vulgaris*. The mutant lacking the *gap* gene was unable to grow with gluconeogenic carbon sources (i.e. succinate), and showed a very reduced growth with glycolytic C sources (i.e. glucose). However, it was able to grow when provided with both types of C sources at the same time. In symbiosis with *Phaseolus vulgaris*, the Gap$^-$ mutant was only able to form small white nodules with no capacity of nitrogen fixation. All free-living and symbiotic mutant phenotypes were reverted after genetic complementation with a wild-type *gap* gene. Complementation was not possible with a *gap* gene version carrying a single nucleotide mutation affecting the Gap active site. The *R. etli* Gap$^-$ mutant was also complemented with *P. syringae gap1* and *gap2* genes, and most of the deficient phenotypes were improved. *gap1* allowed growth of the mutant with unique carbon sources like the wild-type strain, as well as recovery of wild-type nitrogen-fixing efficiency in symbiosis with common beans. However, Pto *gap*2 only improved the ability of AC1D to grow with gluconeogenic, but not with glycolytic C sources. In contrast to *gap1* complementation, expression of *gap2* in the rhizobial AC1D mutant only allowed for partial, yet incomplete recovery of the wild-type nitrogen fixation levels.

The results show the importance of the GAPDH enzyme activity for *R. etli* for free-living and in symbiosis with *P. vulgaris*. The data suggest that both glycolytic and gluconeogenic GAPDH activities are essential for nodulation and nitrogen fixation.

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S6-P-01
Pseudomonas putida KT2440 type VI secretion systems mediate adaptation to the rhizosphere

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The Type VI secretion system (T6SS) is a bacterial nanomachine involved in interbacterial competition. Attacking T6SS$^+$ bacteria release toxins inside prey cells, inhibiting the growth and/or killing competitors in a contact-dependent manner, providing fitness advantages. The biocontrol agent *P. putida* efficiently uses the T6SS as a mechanism to protect plants from deleterious phytopathogens[1]. The strain KT2440 encodes three type VI secretion systems (K1-, K2- and K3-T6SS) but only the K1-T6SS has been proved to be functional and to have antibacterial activity up to date[1]. Here, we study the functionality of the K2- and K3-T6SSs by testing the capacity of single, double and triple T6SS mutants to kill different preys in competition assays. The triple mutant was less competitive than the mutant lacking only the K1 system but only if the prey was a plant pathogen and not a lab strain. This data indicates that the K2- and/or K3-T6SS might be active in *P. putida* natural niches in the presence of real competitors. An increasing number of studies are showing the relevance of T6SS to modulate complex polymicrobial communities, especially in the gut[2]. Here, we study the role of *P. putida* T6SSs in shaping microbial communities in its habitat, the rhizosphere. First, we investigate the capacity to colonise the rhizosphere of tomato plants growing in agricultural soil by the wildtype and the T6SS mutants as previously reported[3]. Secondly, we analysed the microbiome present in the rhizosphere of these plants inoculated with the aforementioned strains. We observed that all T6SS mutants have a lower capacity to colonise the rhizosphere when compared to the wildtype strain. In accordance with that, the Principal Component Analysis (PCA) of the tomato plants microbiota showed two clearly differentiated groups. On one hand, the microbiota of plants inoculated with the wildtype strain that it is able to outcompete foes in a T6SS dependent-manner; on the other hand, the microbiota of plants inoculated with the T6SS mutants unable to do that. These results suggest that the T6SSs of *P. putida* are functional in the rhizosphere, in the presence of competitors, modulate this polymicrobial community and are instrumental for *P. putida* colonization of the plant roots.

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S6-P-02
The *VgrG5a* cluster associated to the type VI Secretion Systems in *Pseudomonas fluorescens* F113 mediate bacterial killing

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The Type six secretion system (T6SS) has been originally described in *Vibrio cholerae* and *Pseudomonas aeruginosa*, as a protein nanomachine system that translocates specific proteins directly into target cells. T6SSs are present in more than 25% of gram-negative bacteria, mostly confined to the phylum Proteobacteria and many of them encoding more than one T6SS in their genome. It has been described that its relevance resides mainly within its anti-prokaryotic activity. The T6SS in *Pseudomonas* has been involved in biofilm formation and anti-bacterial toxins production[1]. The model organism of this study, *Pseudomonas fluorescens* F113 was isolated from the sugar-beet rhizosphere and contains three T6SS in its genomic sequence[2]. Generally, the core genes of the T6SSs are located in genomic clusters, which encode the structural proteins and can include accessory proteins involved in regulation of the T6SSs. Moreover, genes encoding T6SS effectors and their cognate Effector-immunity (EI) pairs proteins are commonly linked to hcp and/or vgrG genes within T6SS clusters. Genes encoding orphan VgrG proteins, not genetically linked to any T6SS structural cluster have also been described for a long number of T6SS-harbouring bacteria[3]. We have identified an orphan *vgrG* islands in F113 (*vgrG5a*) associated with genes encoding putative T6SS-related proteins: a possible regulatory Tap protein, followed by an effector, Tfe8 and an immunity protein, Tfi8. The genetic organization of the region suggests that *tfe8* and *tfi8* may be co-transcribed. In order to test the possible antibacterial activity of this region, two insertional mutants were constructed, one affecting *vgrG5a* and another affecting the *tfe8* gene. A bacterial competition assay revealed that both mutants were affected in their capacity of killing *E. coli*. To test whether Tfe8/Tfi8 constitute an effector-immunity pair, the genes encoding Tfe8 and Tfi8 were cloned in the pT7-7 vector, as a result, the *E. coli* strain expressing *tfe8* and not *tfi8* was affected in growth. These results indicate that Tfe8 is a bacterial killing effector, while Tfi8 is its cognate immunity protein. Tfe8 may represent a novel type of T6SS effector with homology to protein family involved in drug extrusion.

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S6-P-03
Unravelling the non-coding transcriptome of *Sinorhizobium fredii* HH103

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Sinorhizobium fredii HH103 is a broad host range rhizobial strain able to establish nitrogen-fixing symbioses with dozens of legume genera, including the relevant crop soybean. Our research group has extensively characterized the production of bacterial molecular signals (Nod factors, type 3 secreted effector proteins, and different surface polysaccharides) and studied their regulation at the protein-dependent gene transcription level (Pérez-Montaño *et al*. 2016; Acosta-Jurado *et al*. 2019,2020). These studies have included different RNAseq analyses but are clearly incomplete since the analysis of the role of small non-coding regulatory RNAs (sRNAs) has been overlooked.

Recently, we have started the analysis of the non-coding transcriptome of *S. fredii* HH103. For that purpose, we have mixed the total RNA obtained in 15 different conditions and carried out Cappable-seq, which allows the determination of the transcription start sites (TSS) that were active in those conditions. TSSs were associated to messenger RNAs (mRNAs), sense, antisense, or *trans* sRNAs according to their location with respect to the annotated ORFs. The same approach is being undertaken in parallel in another rhizobial strain, *Rhizobium tropici* CIAT899, which enabled reannotation of the two genomes to include the non-coding RNA genes.

In addition, we have performed strand-specific RNAseq on total RNA from bacteria in 6 different conditions: minimal medium (MM, control), MM + mannitol 400 mM (osmotic stress), MM+ genistein (effective nod-gene inducer), and bacteroids of three different HH103 host plants (*Glycine max*, *Lotus burttii*, and *Glycyrrhiza uralensis*). The analysis of these data will allow a more precise characterisation of the specific HH103 transcriptomes for each condition, including protein-coding sequences and sRNAs. We have carried out similar experiments in *R. tropici* CIAT899 and in the model rhizobial strain *Sinorhizobium meliloti* 1021. The comparison of the non-coding transcriptomes of these three rhizobial strains will allow obtaining their catalogues of sRNAs and finding common and specific sRNAs that may be relevant for symbiosis. We will also provide the preliminary results of our studies on the HH103 sRNAs AbcR1 and AbcR2, widely conserved in rhizobia, and F6, unique to sinorhizobial species nodulating soybean.

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S6-P-04
Effect of non-saline osmotic stress on production of Nod factors and other traits of *Sinorhizobium fredii* HH103

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Rhizobia are soil proteobacteria that can establish a symbiotic relationship with host legumes. This interaction requires a complex molecular dialogue in which bacterial NodD proteins play a very important role activating the expression of symbiotic genes, when in the presence of appropriate flavonoids. Among rhizobial symbiotic genes, *nod* genes are responsible for the synthesis of signals molecules called nodulation factors (NF), that are crucial for establishment of symbiosis. Interestingly, in *Rhizobium tropici* CIAT899, besides flavonoids, another factors, like osmotic or saline stress, can activate *nod* gene expression (Pérez-Montaño *et al*., 2016; del Cerro *et al.*, 2019).
We have investigated whether osmotic stress has also an effect on another rhizobial strain, *Sinorhizobium fredii* HH103 (Margaret *et al.*, 2011). In this communication we show that the presence of mannitol 400 mM affects the expression of hundreds of genes, as assessed by RNAseq, and affects different bacterial traits such as motility, and production of exopolysaccharide, NF, acyl homoserine lactones and indole acetic acid (IAA).

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S6-P-05
Characterisation of a novel type 3 secretion system (T3SS) effector from *Sinorhizobium fredii* HH103

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Sinorhizobium fredii HH103 is a nitrogen-fixing bacterium able to nodulate a broad range of legumes that possesses a functional type 3 secretion system (T3SS). The T3SS is one of the bacterial mechanisms involved in the establishment of the rhizobium-legume symbiosis, together with Nod factors and surface polysaccharides (López-Baena *et al*., 2016). The T3SS is a specialized secretion apparatus which delivers proteins, called effectors (T3E), directly from the cytoplasm of the bacterium to the cytoplasm of the host plant cell. The T3E influence nodulation in a positive, neutral and even negative way, being involved in host-range determination and nodulation efficiency. Moreover, the T3SS and the nodulation genes are coregulated in rhizobia, whose transcription is flavonoid and NodD dependent (Teulet *et al*., 2022).
In this work, we studied a novel T3E from HH103, Sfe1. This effector, originally identified *in silico*, shows homology to a family of effectors that has only been identified in phytopathogenic bacteria. In this work, we show that the gene coding this T3E is expressed in minimum medium (MM) induced with the flavonoid genistein. Secretion assays in these inducing conditions were performed to confirm that this protein is secreted by the *S. fredii* HH103 T3SS.

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S6-P-06
Fine-tuning of the *Sinorhizobium meliloti* nitrogen stress response by the non-coding RNA NfeR1

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Nitrogen-fixing root nodule symbioses between rhizobia and legume plants are built on a strict metabolic cooperation between the partners. The nitrogen status of both bacteria and plant is a major metabolic signal that rewires rhizobial gene expression and metabolism during nodulation and symbiotic nitrogen fixation (Patriarca *et al.* 2002). To date, genetic and metabolic reprogramming of rhizobia during the symbiotic transition has been studied almost exclusively from the perspective of the transcriptional control orchestrated by proteins. However, post-transcriptional regulation of gene expression by small RNAs (sRNAs) is expected to play major roles in the establishment of these mutualistic symbioses. A large class of sRNAs are the so-called *trans*-sRNAs that are differentially expressed from intergenic regions and most commonly modulate translation and/or stability of their target mRNAs by short and discontinuous antisense interactions and ribonucleases recruitment (Robledo *et al.* 2020).

The Nodule Formation Efficiency Regulator NfeR1 is the only *trans*-sRNA characterized to date whose loss-of-function compromises nodulation kinetics, nodule development and symbiotic efficiency of *Sinorhizobium meliloti* on alfalfa roots. However, its function has not been delineated with detail yet. This *trans*-sRNAs has three unpaired anti-Shine-Dalgarno (aSD) motifs with redundant regulatory functions, which likely act as interaction seeds for mRNA targeting to block translation (Robledo *et al.* 2017).

We have investigated the transcriptional regulation of *S. meliloti* NfeR1 and we have demonstrated that NfeR1 is a nitrogen stress-induced sRNA, which is transcribed from a dual-mode promoter activated by LsrB (LysR-type symbiotic regulator) and repressed by the master regulator of the nitrogen stress response (NSR), NtrC. LsrB promotes a seemingly constitutive NfeR1 transcription, which is downregulated by NtrC-mediated repression under nitrogen surplus conditions.

The NtrBC two-component system is at the core of regulation of nitrogen assimilation in free-living rhizobia, which is fully active at the onset of nodulation, i.e., nodule formation is totally inhibited by nitrogen excess in soil. We have also demonstrated that NfeR1 is involved in the fine-tuning of the bicistronic mRNA *ntrBC* by direct *ntrB* targeting, which places this sRNAs at the core of the *S. meliloti* NSR regulation. This novel RNA element described here would guarantee the robust feed-back regulation of the NtrBC two-component system, thereby strengthening the NSR by relieving the (auto)repression of the *ntrBC* and helping *S. meliloti* to competitively survive the nitrogen stress of the rhizosphere environment.

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S6-P-07
Sinorhizobium meliloti RNase III: impact in the dynamics and activity of small non-coding RNAs

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Ribonucleases (RNases) are key elements of post-transcriptional regulatory networks that are poorly characterized in rhizobia. Decay of mRNA upon antisense interaction with regulatory small non-coding RNAs (sRNAs) commonly involves prevalent prokaryotic endoribonucleases like RNase III, which is specific to double-stranded RNAs (dsRNA) (Robledo *et al*. 2020; Quendera *et al*. 2020). We previously characterized *S. meliloti* RNase III (SmRNase III) biochemically and genetically (Saramago *et al*., 2018). Here, we analyzed *Sm*RNAse III-dependent alterations of the *S. meliloti* transcriptome under oxic and microoxic conditions, the latter mimicking the symbiotic environment within root nodules.

RNA-Seq revealed a strong impact of this RNase in the *S. meliloti* transcriptome as expected from the pleiotropic phenotype of the knock-out mutant. Protein-coding genes misregulated in the mutant with respect to the wild-type strain ($log_2FC > 1$ or < -1 in the combined aerobic and microaerobic transcriptome) represent 42% of ORFs annotated in the *S. meliloti* genome. We further correlated alterations in the steady-state levels of mRNAs with those of their corresponding antisense sRNAs (asRNAs) and compared the *Sm*RNase III-dependent transcriptome with the mRNA interactomes of the well-characterized AbcR1 and AbcR2 *trans*-sRNAs.

This analysis anticipates a great impact of this endoribonulcease in the post-transcriptional RNA silencing of genes relevant to both the free-living and symbiotic rhizobial lifestyles.

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S6-P-08
Wild soybean cultivar specificity is associated to the symbiotic T3SS

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Glycine soja (Siebold and Zucc.) is the wild ancestor of the domesticated soybean (*G. max*). During soybean domestication, many natural phenotypic changes affecting plant development, seed size and protein and oil content, among others, have occurred. In this process of domestication, traits controlling the formation of symbiotic root nodules by several host resistance (*R*) genes, referred to as *Rj/rj* genes, have been maintained in agronomically improved soybean cultivars. These R proteins interact with type 3 secretion system effectors (T3E), blocking nodulation. Four *R* genes have been traditionally considered in soybean as associated to nodulation restriction: **i**) the *Rfg1* gene, which restricts nodulation with some *Sinorhizobium fredii* strains such as USDA257, USDA205, and USDA193; **ii**) *Rj2, a*n allelic variant of *Rfg1*, which restricts nodulation with *Bradyrhizobium japonicum* USDA122; **iii**) Rj3 soybeans cannot be nodulated by some *B. elkanii* strains such as USDA33, BLY3-8, or BLY6-1; and **iv**) strains such as *B. japonicum* Is-34 or *B. elkanii* USDA61 cannot nodulate Rj4 soybeans. Recently, a new R protein, GmNNL1, has been described to interact with the *B. diazoefficiens* USDA110 NopP to inhibit nodulation during root hair infection.

Previous studies of our research group indicate that inactivation of the *S. fredii* T3SS can block or induce the formation of nodules in several *G. soja* accessions. This suggests that wild soybeans could have R proteins that interact with still unknown *S. fredii* T3E or even possess new resistance proteins, or R proteins with different sequences, that could be the origin of the cultivar specificity phenotype in agronomically improved soybeans.

In this work we studied whether the *S. fredii* genes involved in wild soybean nodulation specificity are associated to the symbiotic T3SS and whether it is possible to modulate nodulation range by transferring symbiotic plasmids from *S. fredii* strains able to nodulate with soybeans and wild soybeans to strains unable to nodulate with these legumes. Our final goal is to find new R proteins in wild soybeans that interact with T3E, determine if they are conserved in domesticated soybeans, and study their evolution during soybean domestication.

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S6-P-09
Sinorhizobium fredii HH103 surface motility is induced by flavonoids and the NodD1 and TtsI bacterial regulatory proteins

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Bacteria can move on surfaces to colonize new environments and get more resources. Rhizobia are soil proteobacteria able to establish a symbiotic nitrogen-fixing interaction with legumes relying on a complex signal interchange between both partners. The interaction between the flavonoids exuded by legumes and the bacterial transcriptional activator NodD regulates the transcription of different rhizobial genes (the so-called *nod* regulon) and, with the participation of additional bacterial regulatory proteins (such as TtsI, MucR or NolR), influence the production of different rhizobial molecular signals. In *S. fredii* HH103, a broad host-range rhizobia able to nodulate dozens of legume genera including soybean, the *nod*-gene inducer flavonoid genistein and NodD1 trigger the production of Nod Factors as well as the type 3 secretion system (T3SS) assembly and the subsequent effector proteins secretion, but repress the exopolysaccharide production and biofilm formation (Acosta-Jurado et al. 2016; Pérez-Montaño et al. 2016). In this communication, we report that genistein promotes a surface translocation which involves both flagella-dependent and independent mechanisms, but not affects swimming motility. This surface motility is regulated in a flavonoid-NodD1-TtsI-dependent manner, relies on the assembly of the symbiotic T3SS, and involves the participation of additional modulators of the *nod* regulon (NolR and MucR1) (Alías-Villegas et al. 2022). To the best of our knowledge, our investigations show for the first time in a rhizobial strain that the inducer flavonoids can activate both T3SS synthesis and surface motility.
We will also show that the *S. fredii* HH103 SFHH103_00346-SFHH103_00348 genes, whose expression is driven by a previously unknown and not fully conserved *tts* box (a rhizobial promoter sequence where TtsI, the main regulator of T3SS, binds and activates the gene expression), is involved in genistein-induced surface motility. The inactivation of these genes affects swimming motility and also partially impairs the symbiosis with soybean.

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S6-P-10
Rational reconstruction of a broad host range vector to overexpress heterologous proteins *in vivo*

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Protein overexpression systems have been traditionally used to study the role of a determined protein in diverse cellular processes, as a complement to the construction of bacterial mutants lacking such protein. These systems have proven their value in bacterial cultures, or co-cultures of bacteria and higher eukaryotes, to increase the concentration of a given protein over the course of the assay. However, one of the main challenges limiting their usefulness for *in vivo* application, as bacterial colonization assays, is the protein leakage due to basal expression of the promoters used. This may lead to misinterpretations of experiments when the protein is not expressed in an appropriate moment or place during the infection. Moreover, the potential toxicity to bacterial hosts and the high cost of the inducer molecule (e.g. IPTG or tetracycline) might restrict the application of some expression systems. Furthermore, these systems are not usually conceived to be used in different bacterial species, and therefore the stability of plasmid vectors bearing regulatory elements can be compromised in some bacterial hosts, in the absence of selective pressure. Here we describe the redesign of a protein expression system that was used in the past for heterologous protein overexpression, along the progression of diverse infections (Medina C *et al*., 2011; Medina C *et al.*, 2012). We have combined in the same DNA fragment the salicylate-inducible cascade expression system, composed by a regulatory module containing the two divergent promoters P*nah*/P*sal* that drive the expression of *nahR* and *xylS2-nasR* respectively, with a constitutively expressed *dtomato* reporter gene and an expression module composed by the Pm promoter tailed by a multiple cloning site. The system is almost silenced by the presence of a transcriptional attenuator upstream of the Pm promoter that is anti-terminated upon the addition of the non-toxic molecular inducer salicylate. This construction has been cloned in the vector pFAJ1702 that is stable in a wide background of gram-negative bacteria, by the presence of the post-segregational killer loci from the symbiotic plasmid of *Sinorhizobium fredii* sp. NGR234 (Dombrecht *et al*., 2001). This vector was introduced in rhizobacteria such as *Azospirillum* sp, *Pantoea* sp, *Pseudomonas putida* and several rhizobial strains, and its stability and inducer conditions were tested, demonstrating its usefulness under laboratory conditions.

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S6-P-11
Fine-tuning modulation of oxidation-mediated posttranslational control of *Bradyrhizobium diazoefficiens* FixK2 transcription factor

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$FixK_2$ is a CRP/FNR-type transcription factor that plays a central role in a sophisticated regulatory network for the anoxic, microoxic and symbiotic lifestyles of the soybean endosymbiont *Bradyrhizobium diazoefficiens* (reviewed in Salas et al., 2021). Apart of the balanced expression of the *fixK*$_2$ gene under microoxic conditions (induced by the two-component regulatory system FixLJ and negatively auto-repressed), $FixK_2$ activity is posttranslationally controlled by proteolysis, and by oxidation of a singular cysteine residue (C183) near its DNA-binding domain (reviewed in Fernández et al., 2016).

To simulate permanent oxidation of $FixK_2$, we replaced C183 for aspartic acid. This semi-conservative replacement (due to both its size and charge) would mimic $FixK_2$ overoxidation (sulfenic/sulfinic acid cysteine derivatives). Purified C183D $FixK_2$ protein showed both low DNA binding and *in vitro* transcriptional activation from a genuine $FixK_2$ target, i.e., the promoter of the *fixNOQP* operon (Cabrera et al., 2021), which is required for respiration under symbiotic conditions. However, in a *B. diazoefficiens* strain coding for C183D $FixK_2$, expression of a *fixNOQP'-'lacZ* fusion was similar to that in the wild type, when both strains were grown microoxically. The C183D $FixK_2$ encoding strain also showed a wild-type phenotype in symbiosis with soybeans, and increased *fixK*$_2$ gene expression levels and $FixK_2$ protein abundance in cells. These two latter observations together with a global transcriptional profile of the microoxically cultured C183D $FixK_2$ encoding strain suggest the existence of a finely tuned regulatory strategy to counterbalance the oxidation-mediated inactivation of $FixK_2$ *in vivo*.

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S6-P-12
Sinorhizobium meliloti type IVc pili: exploring their role in surface-associated behaviors and plant colonization

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Type IV pili are long thin microbial appendages, which are formed by thousands of proteins known as pilins. Through cycles of extension and retraction resulting from pilin polymerization and depolymerization, respectively, pili serve different functions in bacteria including surface sensing, attachment and biofilm formation, motility across surfaces and host colonization (Ellison et al. 2022). Despite their relevant role in diverse microbial lifestyles, knowledge about these appendages in rhizobia is still limited.

The *S. meliloti* genome harbors several genes coding for proteins known to participate in the assembly of type IVc pili (T4cP) also known as Flp (Fimbrial low-molecular-weight proteins) or Tad (Tight adhesion) pili. In the reference strain Rm1021, T4cP genes are organized in two clusters: flp-1, which is located on the chromosome and flp-2, which is located on the pSymA megaplasmid and seems to be truncated. Bundle-forming pili associated to the chromosomal flp-1 region have been involved in competitive nodulation of alfalfa plants (Zatakia et al. 2014). However, no information is available about the role of the flp-2 region in the biogenesis of pili and their function.

In the highly competitive strain *S. meliloti* GR4, genes that are absent in Rm1021 and potentially code for pilus assembly proteins, have been identified in its pSymA megaplasmid. In this study, we have investigated the role of the flp-1 and flp-2 regions in the production of T4cP in GR4. Single and double flp mutants have been constructed in the wild-type genetic background as well as in flagella-less derivative mutant strains. The different resulting strains have been assessed for pili production by Transmission Electron Microscopy observations. The role of these appendages in surface-associated behaviors, plant colonization and nodulation will be presented.

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S6-P-13
Production of cellulose and MLG β-glucans by *Rhizobium etli* CFN42

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Bacterial exopolysaccharides (EPS) are secreted biopolymers with important roles in bacterial survival, colonization and interaction with eukaryotic hosts. Among the large diversity of EPS are the bacterial linear β-glucans, unbranched polysaccharides formed by D-glucose units linked by β-glycosidic bonds, which include curdlan (β 1→3 D-glucose), cellulose (β 1→4 D-glucose) and the more recently described Mixed Linkage β-Glucan (MLG; (β 1→3 β 1→4 D-glucose). Bis-(3',5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is a universal bacterial second messenger involved in the bacterial decision to attach to a surface and form a biofilm community. C-di-GMP is a key activator of the production and secretion of different biofilm matrix components, including diverse EPS. In this work we report the common bean symbiont *Rhizobium etli* as the first bacterium capable to produce the β-glucans cellulose and MLG. Significant amounts of these two β-glucans are not produced in free-living laboratory conditions, but their biosynthesis is triggered when intracellular c-di-GMP levels are elevated. Both β-glucans contribute to Congo red (CR^+) and Calcofluor (CF^+)-positive colony phenotypes. Cellulose appears to be more relevant than MLG for aggregation and biofilm formation under high c-di-GMP conditions. None of these two EPS are essential for attachment to roots of *Phaseolus vulgaris*, neither for nodulation nor for symbiotic nitrogen fixation. However, they separately contribute to the fitness of interaction between *R. etli* and its host. Overproduction of these β-glucans, particularly cellulose, appears detrimental for symbiosis. This indicates that their biosynthesis must be strictly regulated in time and space. Although both cellulose and MLG production are activated by c-di-GMP, it is likely that their biosynthesis is controlled by different, yet unknown regulatory pathways.

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S6-P-14
Metagenomic insights into soil microbial communities after sowing legume-rich mixtures in the dehesa

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The dehesa (Spain) and montado (Portugal) are traditional systems in oak savannas that sustainably integrate agriculture, livestock and forestry (Plieninger *et al*., 2015). The pastures in these systems are increasingly sowed with legume-rich mixtures to increase their productivity and quality (Moreno *et al*., 2018). Recently, it has been shown that sowing legumes has an important effect on soil microbial community structure and composition (Moreno *et al*., 2021). However, if this translates to changes in microbial functionality is not known. This is important because the activity of the soil microbiome influence a large number of important ecosystem processes, including nitrogen, phosphorus and carbon cycling. In this study, we assess, using shotgun metagenomics, the impact of sowing legumes (≤ 5 or >10 years) on the microbial functional potential related to these three cycles. We found that sowing legumes has a profound impact in both the diversity and composition of N, P and C cycling genes. For example, the relative abundance of *nifH* genes decreased significatively in soils planted with legumes. Whether or not these genetic changes affect different ecosystem processes, such as carbon and nutrient cycling, is currently being investigated.

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S6-P-15
Consensus identification and symbiotic transcriptional profile of ethylene biosynthesis genes in *Medicago truncatula*

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The gaseous hormone ethylene is a well-known inhibitor of symbiotic legume-rhizobium interactions. Interestingly, ethylene biosynthesis has been shown to occur in several legumes hours after rhizobium inoculation in a Nod-factor dependent manner. However, both the molecular mechanisms underlying this process and the specific ID of the enzymes responsible for this biosynthesis remain largely unknown.

In plants, ethylene biosynthesis is mediated by the action of two consecutive enzymes, 1-aminocyclopropane 1-carboxylate synthase (ACS) and oxidase (ACO). In contrast to other plant species, in the model legume *Medicago truncatula* these families have not been described in detail. This leads to misinterpretation of scientific results relying only on BLAST-based annotations. Furthermore, they are quoted in the literature using different nomenclature, complicating matter further.

The objective of the current work is two-fold; first, we have carried out a comprehensive analysis of the latest *M. truncatula* genome version (v. 5.1.8, Pecrix et al., 2018), performing a combination of reciprocal BLAST and phylogenetic analysis to revaluate the annotation of all *ACS* and *ACO* genes. In the case of the ACS family, we discovered two genes wrongly annotated as *ACS*, which belong to the family of aminotransferases, and numerous annotated as *ACOs* which belong to the 2-oxoglutarate and Fe(II)-dependent oxygenase superfamily. This has led to the identification and consensus naming of a total of 9 ACSs and 5 ACOs. Second, we have performed a meta-analysis, compiling the existing RNA-seq transcriptomic data for different *M. truncatula* tissues and conditions, with a special focus on symbiosis. Finally, we have confirmed the expression of some of these genes using quantitative real-time PCR and promoter:GUS fusion analyses.

This dataset establishes a framework upon which to propose candidate genes responsible for ethylene biosynthesis, both during the infection process, at early symbiotic stages, and in mature nitrogen-fixing nodules, for future investigation.

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S6-P-16
Azospirillum brasilense Ab-V5 recruits maize rhizosphere and bulk soil microbiome to stimulate plant growth promotion

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Plant growth promoting bacteria (PGPB)-based biostimulants have been used as alternative and integrative inputs to minimize the use of mineral fertilizers and pesticides in agriculture. The genus *Azospirillum* has shown potential in plant growth promotion for maize and other crops[1]. However, the lack of holistic comprehension about PGPBs-plant-native soil microbiome can lead to inconsistency of results in field conditions. Recent ecological theories revealed that plant microbiomes are organized as communities with keystone species and helpers which, in synergism, can impact plant health and productivity[2,3]. Thus we aimed to characterize the microbial community associated with maize under influence of a native soil (NS) microbial community abundance gradient and the PGPB maize inoculant *A. brasilense* Ab-V5. Our hypothesis was that PGP efficiency is related to Ab-V5 niche occupation and persistence, and this could be a proxy for enrichment of other beneficial soil-plant bacteria groups in the rhizosphere. Aiming to understand the PGPB-plant-microbiome system, we conducted a greenhouse experiment in which the synergistic effect of Ab-V5 and soil native microbial communities (NS, NS dilutions 10^{-3}, 10^{-6} and 10^{-9}, and irradiated soil-IS) was evaluated through plant phenomics, monitoring 16S rRNA gene and Ab-V5 genome-equivalent abundance by qPCR, bulk soil and rhizosphere 16S rRNA metataxonomics, metagenomics and data integration. We constructed microcosms containing Gamma [^{60}Co] irradiated pot soil to drastically reduce starting microbial inoculum and a dilution-to-extinction gradient of a maize field soil (collected 5-10 cm bellowground) was transplanted to the irradiated soil, with four replicates each. NS and IS soils were used as controls. Our results revealed that synergism of NS 10^{-3} + Ab-V5 and NS 10^{-6} + Ab-V5 promoted higher means of shoot and root dry weights, root length and root volume compared to NS + Ab-V5. The abundance of 16S rRNA gene was not statistically different among treatments in bulk soil after 15 days of sowing (DAS). In treatments NS 10^{-3} and NS 10^{-6}, Ab-V5 persists detectably in the maize rhizosphere until 15 DAS with higher abundances compared to NS. We identified potential keystone genera in bulk soil and rhizosphere communities through metataxonomics analysis, specially plant-soil beneficial bacteria in NS 10^{-3} + Ab-V5. Genes involved in bacterial metabolism of carbon compounds, denitrification, tryptophan metabolism and transcriptional regulation were more abundant in the NS 10^{-3} + Ab-V5 plant rhizosphere, indicating that these pathways were key for plant growth promotion. In NS + Ab-V5, higher abundance of *Sphingomonas* and *Bradyrhizobium* were positively correlated with genes involved in degradation of xenobiotic compounds. Our results indicate that Ab-V5 abundance in microbiota is dependent on microbial interactions of the biostimulant with plant-associated native microbial communities. This multi-omics approach for the holistic view of the complex system composed by PGPB-plant-microbiome opens new perspectives for optimization of biostimulants formulation and recommendation with enhanced efficiency for plant growth promotion.

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S6-P-17
Dissecting the role of extracellular membrane vesicles in the molecular dialogue between plant host and rhizobial strains

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Rhizobia are a group of α- and β-proteobacteria that have the ability to establish symbiosis with legumes. In this process, rhizobia infect legume roots and induce the formation of a new type of organs, the nodules. Eventually, rhizobia invade nodule cells, where they differentiate into bacteroids, a form that expresses nitrogenase genes, fixing N_2 into ammonia. The bacteroids then supply the ammonia to the plant that, in exchange, supplies the bacteroids with carbon and other nutrients (1). The entire process is known as nodulation and it is highly relevant since it promotes plant growth having the potential to replace polluting chemical-based fertilizers, which negatively impact the environment and contribute to global warming.

This symbiosis is markedly specific, since each rhizobial strain can only nodulate with specific host legumes. *Sinorhizobium fredii* HH103 is one of the best studied rhizobia. This strain is a broad-host range rhizobium, being able to nodulate dozens of legumes, including essential crops as *Glycine max*, the major crop legume needed for the production of human food, animal feed, and biofuel, which makes this strain an agronomical relevant rhizobium.

The rhizobia-legume symbiosis is established by a complex molecular dialogue that starts with root exudates that include flavonoids, phenolic compounds that activate the NodD protein, a transcriptional regulator that induces the expression of nodulating genes, and therefore, the Nod factors production. These signal molecules produce different responses in the plant that lead to bacterial infection and nodule formation (2).

In order to deepen our understanding of how this molecular dialogue works further molecular players must be considered. Recently, attention has been drawn to extracellular vesicles, as they are membranous vehicles that convey DNA, siRNA, proteins, signal molecules, and metabolites between the plant and the rhizobium. By these means, extracellular membrane vesicles shape interkingdom interactions modulating the plant and rhizobial responses throughout the different stages of plant growth and nodule development (3). In previous studies it has been observed that extracellular vesicles carry Nod factors. In this work we endeavor to dissect the molecular dialogue mediated by extracellular membrane vesicles employing a holistic a multi-omic approach to determine the vesicle cargo. In order to perform these analyses, we isolated the vesicles from free-living rhizobia. We pipelined the workflow to purify rhizobial vesicles by means of ultrafiltration and ultracentrifugation techniques. We will evaluate the impact of flavonoids supply upon vesiculation rates as well as the differential cargo packaging in rhizobial strains. Subsequently, we will pinpoint important players present in the cargo of such membrane vesicles during the nodulation process by generating a set of knockout mutants. Finally, we will assess nodulation improvements when supplying vesicles derived from flavonoid-treated and untreated strains as well as mutants constructed in the previous step onto differentially matured nodules.

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