

Rhizosphere 5



ABSTRACT BOOK

July 7 - 11, 2019

Saskatoon, Saskatchewan, Canada

Sunday, July 7th, 2019

15:30 - 16:45

OPENING CEREMONY

(SALON B+C)

16:45 - 17:45

KEYNOTE ADDRESS - LEON KOCHIAN

(SALON B+C)

SHINING A LIGHT ON RHIZOSPHERE RESEARCH IN SASKATCHEWAN

L Kochian¹

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When I came to the University of Saskatchewan from Cornell University 2 ½ years ago, I was very impressed by the breadth and depth of rhizosphere research being conducted at the university. In my presentation, I will not only present findings from my own program on cereal crop (sorghum and maize) adaptation to acid soils that limit crop production on up to 40% of the world's arable land, particularly in the developing countries in the humid tropics, but also will present a look at some of the interesting rhizosphere research being done by other USask researchers. This includes work on the root microbiome, including the impact of the root microbiome on carbon recycling in the soil, research on fungal endophytes that improve crop tolerance to soil-based abiotic stresses, and research at the Saskatchewan Centre for Cyclotron Research using a recently developed plant PET imaging system (the only one in Canada) to visualize and quantify photoassimilate translocation from the shoot to the root and out into the rhizosphere. For research from my long-time collaboration with Embrapa Maize and Sorghum in Brazil, I will present findings based on high throughput phenotyping of sorghum root growth and architecture and the subsequent genetic mining of phenotype by genotype data to identify sorghum genes that confer tolerance to the two limiting factors that greatly reduce crop yields on acid soils: aluminum toxicity and low phosphorous availability. The Al tolerance genes underlie a system mediating exudation of citrate from the root apex, where it chelates and detoxifies rhizotoxic Al³⁺ in the rhizosphere. The P efficiency genes identified by association genetics analysis of candidate genes, appear to alter sorghum root architecture, allowing the larger and finer root system to more effectively "mine" phosphate anions fixed to clay particles in the acid soil.

17:45 - 20:00

WELCOME RECEPTION

(FOYER & SALON A, D, E)

08:30 - 09:30 **KEYNOTE ADDRESS - TIINA ROOSE** **(SALON B+C)**

MULTISCALE IMAGE BASED MODELLING OF RHIZOSPHERE PROCESSES

T Roose¹

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In this talk I will describe a state of the art image based model of the soil-root interactions, i.e., a quantitative, model of the rhizosphere based on fundamental scientific laws. This will be realised by a combination of innovative, data rich fusion of structural imaging methods, integration of experimental efforts to both support and challenge modelling capabilities at the scale of underpinning bio-physical processes, and application of mathematically sound homogenisation/scale-up techniques to translate knowledge from rhizosphere to field scale. The specific science question I will address with these techniques is how to translate this knowledge from the soil pore and single root scale to root system, field and ecosystem scale in order to predict how the climate change, different soil management strategies and plant breeding will influence the soil fertility.

09:30 - 10:00 **POSTER PITCHES** **(SALON B+C)**

10:00 - 10:30 **HEALTH BREAK & POSTERS & EXHIBITS** **(FOYER & SALON A, D, E)**

10:30 - 12:30 **PARALLEL SESSIONS**

SESSION 1: THE ROOT MICROBIOME **(GALLERY A)**

Session Chairs: Sue Grayston & Simeon Smail

10:30 - 11:00

PLANT MICROBIOME: UNRAVELLING THE BIOTA BLACK BOX

V Gupta¹, Dr. A Richardson¹

¹*CSIRO Ag&Food, Glen Osmond, South Australia, Australia*

Plant's microbiome, above- and below-ground microbiomes, is an integral part of the wider plant genome and is now considered as the extended phenotype of all plants. The below-ground root-microbiome has been shown to have a major impact on plant health through interactions on growth and development, facilitation of nutrient uptake and ability to tolerate biotic and abiotic stresses. New *omics* tools are helping to unravel the complexities of its enormous diversity and functional potential and there is now new opportunity to develop 'Designer microbiomes'.

Our research targets the identification of key drivers of microbiome diversity and functionality along with understanding of spatial and temporal factors that operate under field conditions. The plant microbiome

assemblage, in terms of composition and abundances of specific functional groups, is both soil type and host species dependent and could be modulated by management. Plants ability for selective recruitment from the general soil microbiome results in significant reductions in species diversity, richness and evenness as the interaction becomes more intimate from the bulk soil to the endosphere. For example, (i) within cereal crops and perennial grass species there is a plant type and variety specific enrichment of specific members of soil microbiome both in terms of total bacteria and diazotrophs, (ii) clear differences in bacterial and fungal microbiomes exist between the domesticated modern bread wheats, landraces and wild relatives within the Triticeae, (iii) a diverse array of microbial communities that are well connected are involved in the continued effective expression of disease suppression in the field environment and (iv) a diverse microbial community exists in the aboveground plant parts potentially contributing to beneficial functions. Overall, the aim is to develop better connection between ‘descriptive genomics’ and ‘functional microbiome’ that would allow deliberate selection of beneficial microbiomes through either targeted management or specific selection of crop genotypes.

11:00 - 11:15

DOES THE SEED MICROBIOME PERSIST IN THE PLANT RHIZOSPHERE OVER TIME?

CL Khodadad¹, M Hummerick¹, AR Dixit¹, CJ Sperrn¹, LE Spencer¹, AB Curry¹, JL Gooden¹, JA Fisher³, JA Polanco⁴, GJ Maldonado Vazquez⁵, MW Romeyn², GD Massa²

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Plant associated microbiomes contain communities of bacteria and fungi that may provide a mutualistic or a pathogenic role with the potential to influence plant development or physiology. These microbes located in both the phyllosphere and rhizosphere can affect plant growth, but little is known about these communities and many questions about community structure and function have yet to be answered. As technology advances and humans travel greater distances from Earth, additional challenges are introduced for growing food crops under space flight conditions. The natural microbiome of the plants grown in closed environments designed for space travel would presumably be subjected to selective pressures as a result of increased atmospheric CO₂ levels, water stress, suboptimal temperatures, and sanitization protocols for seeds and growth media. To address some of these factors, three crops, mizuna (*Brassica rapa var japonica*), red romaine lettuce (*Lactuca sativa* cultivar ‘Outredgeous’), and cherry tomato, (*Solanum lycopersicum* cultivar ‘Red Robin’) were grown under simulated International Space Station (ISS) environmental conditions (RH, CO₂, temperature) and harvested at different developmental stages. Sanitized and unsanitized seeds were germinated, and plants were harvested at 7, 14, 21, and 28 days for leafy greens and 28, 42, and 76 days for the tomato. The number of heterotrophic bacteria and fungi per gram of plant tissue was ascertained by plate counts on appropriate agar media. The seed microbiome and subsequent rhizosphere microbiomes were determined and compared using 16S rRNA gene and ITS sequencing. Bacterial densities varied between sanitized and unsanitized seed

treatments on the leaves of mizuna and lettuce but not the roots. The rhizosphere microbiome among plants was consistent but varied between plant types indicating a unique community for each and a need to expand our limited knowledge of plant associated microbes for different crops.

11:15 - 11:30

DEVELOPMENT OF THE RHIZOSPHERE MICROBIOME IS DEPENDENT ON ROOT AGE AND THE PRECEDING DETRITUSPHERE

Y Zhou¹, MD Denton¹, DR Coventry¹, GV Vadakattu², BN Kaiser³, A Merchant³, D Fuentes³, J Li⁴, Y Wei⁴, H Liu⁵, Y Wang⁵, S Gan⁵

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Microbiomes in the rhizosphere perform important roles in the function and productivity of crops, but the influence of root development or the previous detritosphere is poorly understood. In a series of controlled environment studies, we investigated the changes in root microbiomes during the development of chickpea root systems and measured the importance of a preceding wheat detritosphere in modifying the rhizosphere microbiome. A specialised rhizotron system enabled accurate sampling of specific stages of root development, root exudates, soil porosity and sampling for root microbiome analysis. In total, 128 samples, and 11,169,570 clean sequences of 300 bp were obtained and clustered into 13,236 OTUs. Over 70% variation of microbiome composition was explained by the first two principal components and demonstrated distinct separations of endosphere and rhizosphere microbiomes, and differences related to root ages and genotypes. Rhizospheres had more enriched OTUs than endospheres, but the enriched OTUs depended on soil types. Annotation of OTUs revealed the importance of soil type in determining order level taxonomy: *Actinomycetales* were the dominant order in rhizospheres, *Rhizobiales* were more dominant in endospheres and *Burkholderiales* were dominant in older rhizocompartments. Microbial diversity was influenced by root age, rhizocompartment, soil type and plant genotype; endophytes had lower diversity than rhizospheres and bulk soil. In another study we established an in-depth soil microbial gene catalogue (19.8 million genes and 10.2 Gbp total length) based on the living-decaying rhizosphere niches. The detritosphere microbiome regulated the composition and function of the rhizosphere microbiome to a greater extent than plant type: rhizosphere microbiomes of wheat and chickpea were homogenous (65-87% similarity) under simulated no-tillage management but were heterogeneous (3-24% similarity) under tillage, which disrupted the detritosphere. These studies identified that the age of a root system and the detritosphere were pivotal in defining the rhizosphere microbiome and ultimately influence plant growth, health and productivity.

11:30 - 11:45

PLANT FUNCTIONAL GROUP AND THEIR ASSOCIATED ROOT TRAITS AS PREDICTORS OF THE SAPROPHYTIC FUNGAL COMMUNITIES IN EXPERIMENTAL GRASSLANDS.

D Francioli¹, S van Rijssel², A Termorshuizen³, A Cotton⁴, A Dumbrell⁵, J Raaijmakers², J van Ruijven¹, L Mommer¹

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Saprophytic fungi decompose organic matter and are important agents of soil mineralization and carbon cycling. Their community structure is known to be affected by abiotic conditions like soil pH and moisture, but the extent to which it is driven by plant community composition is not fully understood. Here, we determined the root-associated saprophytic fungal (RASf) community in grassland plant communities, which differed in species richness (1-8 species) and composition, using NGS sequencing. Root and soil samples were collected at two soil depths and several soil characteristics and plant root traits were measured to link these factors to RASf community structure. Across the plant monocultures, plant functional group (grasses vs forbs) was the main factor driving RASf community structure, whereas soil depth did not have a significant effect. Interestingly, lignin content and root C/N ratio, which showed a plant functional type pattern, were related to RASf community structure and explained approximately 11% of community variation. We found that saprophytic fungal richness increased with plant species richness, which may be due to an increase in soil organic matter content (SOM) with plant species richness. However, the saprophyte taxa detected in mixed plots were to a large extent a subset of those found across the monocultures. In the mixed plots plant functional type had the greatest effect (9 % of variation) on RASf community structure, whereas plant species richness and soil depth contributed marginally (less than 5%) to explaining community variation across the different plant communities. This work represents the first in-depth investigation of the root-associated saprophytic fungi in grassland plant communities, identifying plant functional group and their associated root traits as important mediators of saprophytic community structure.

11:45 - 12:00

CONTRASTING LONG-TERM FERTILIZATION REGIMES DIFFERENTIALLY IMPACT ROOT-ASSOCIATED MICROBIAL COMMUNITIES DURING THE DEVELOPMENT OF FIELD-GROWN MAIZE

A Bourceret¹, R Guan¹, S Spaepen², N Gerlach³, M Bucher³, K Dorau³, T Mansfeldt³, P Schulze-Lefert¹

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Intensive farming practices are increasingly applied to feed the world's constantly growing population. The resulting environmental issues mean that there is an urgent need for new practices that allow efficient plant

growth under limited fertilization amendments. The engineering of the microbial communities that interact with plant roots represents one such strategy to augment plant growth and fitness. Thus, the aim of this project is to develop a global approach to assess the impact of root-associated microbes and management practices on plant yield. To this end, maize (*Zea mays* L.), one of the world's most cultivated crops, was chosen as a model to study the impact of the root microbiota on plant yield and fitness under different agricultural management practices and in soils with low nutrient availability.

Using amplicon-based sequencing, we have compared the diversity and structure of bacterial, fungal, and oomycetal communities in soil, root, and rhizosphere samples collected before sowing (unplanted soil), from 7-week-old (vegetative stage) and 15-week-old (reproductive stage) maize plants. Five different plant genotypes were grown in long-term field experiments, located at two distant locations in Switzerland, to compare four different fertilization conditions.

In unplanted soil, the soil biome was shaped by the spatial variability of soil characteristics between and within the two fields. During maize development, plant host and fertilization regimes are the main drivers of root microbiota structure, although a modest but significant plant genotype effect was also observed. Our analysis has allowed us to identify contrasting trends in the different microbial kingdoms during crop cultivation: bacterial communities seem to be more sensitive to natural soil heterogeneity within the field (e.g. pH) than fungal and oomycetal communities, driven mainly by biogeography (field location). This global analysis encompassing the three different microbial kingdoms characterizes the role of microbial interactions in plant fitness in natural soils with varying nutrient availability.

12:00 - 12:15

SOIL-BORNE LEGACIES OF PLANT DISEASE

G Vismans¹, P Goossens¹, CM Pieterse¹, PA Bakker¹, RL Berendsen¹

¹*Utrecht University, Utrecht, Netherlands*

Plants can adjust their root microbiome upon pathogen infection and specifically recruit a group of disease resistance-inducing microbes. As a result, infected plants can create a soil-borne legacy of disease that confers enhanced protection against the pathogen in a second population of plants growing in the same soil. This has been demonstrated with infections of Arabidopsis plants by the bacterial leaf pathogen *Pseudomonas syringae* and such soil-borne legacies are also consistently produced when Arabidopsis plants are infected by the downy-mildew pathogen, *Hyaloperonospora arabidopsidis*. The root-specific transcription factor MYB72 emerged as an important regulator of rhizobacteria-induced systemic resistance (ISR). MYB72 is required for the systemic onset of ISR and it regulates the biosynthesis of coumarins that affect the composition of the root microbiome. We therefore examined the role of MYB72 in both the creation as well as perception of soil-borne legacies. Although the bacterial ISR-model strain *Pseudomonas simiae* WCS417r cannot trigger ISR in *myb72* plants, *myb72* plants are able to perceive a soil-borne legacy created by wild-type downy-mildew-infected plants. This indicates that MYB72 is not essential for the perception of soil-borne legacies and MYB72-independent signaling is involved in the recognition of recruited microbes. However, *myb72* plants infected by *H. arabidopsidis* did not improve the resistance of wild-type plants subsequently growing on the same soil. This indicates that MYB72, and likely coumarins, are required for the creation of soil-borne legacies of plant disease.

We are currently exploring the role of coumarins in selective recruitment of beneficial microbes and the creation of resistance-inducing microbiomes.

12:15 - 12:30

STRUCTURAL EQUATION MODELLING OF A WINNOWED SOIL MICROBIOME IDENTIFIES HOW INVASIVE PLANTS RE-STRUCTURE MICROBIAL NETWORKS

SD Siciliano¹, SD Mamet¹, E Redlick², M Brabant², EG Lamb³, BL Helgason¹, K Stanley²

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The development of microbial networks is central to ecosystem functioning and is the hallmark of complex natural systems. Characterizing network development over time and across environmental gradients is hindered by the millions of potential interactions among community members, limiting interpretations of network evolution. We developed a feature selection approach using data winnowing that identifies the most ecologically influential microorganisms within a network undergoing change. Using a combination of graph theory, leave one out analysis, and statistical inference, complex microbial communities are winnowed to identify the core organisms responding to external gradients or functionality, and then network development is evaluated against these externalities. In a plant invasion case study, the winnowed microbial network became more influential as the plant invasion progressed as a result of direct plant-microbe links rather than the expected indirect plant-soil-microbe links. This represents the first use of structural equation modeling to predict microbial network evolution, which requires identification of keystone taxa and quantification of the ecological processes underpinning community structure and function patterns.

SESSION 2: SPATIO-TEMPORAL ORGANIZATION IN THE RHIZOSPHERE

(GALLERY B)

Session Chair: Doris Vetterlein

10:30 - 11:00

X-RAY CT BASED PLATFORM FOR INTEGRATION OF PHYSICAL, CHEMICAL AND BIOLOGICAL INFORMATION ON SPATIOTEMPORAL PATTERNS AT SINGLE ROOT AND ROOT SYSTEM SCALE

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¹*Helmholtz Centre for Environmental Research - UFZ, Halle, Germany*

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Spatiotemporal organization of the rhizosphere is a key to understand rhizosphere functions. Major challenges are (i) the integration of spatial patterns for physical, chemical and biological parameters in 2D and 3D for systems reflecting radial root geometry and (ii) bridging the relevant scales of local interaction. The latter ranging from nm to mm around a single root, and to cm for the root system perspective.

Within the framework of the priority program “spatiotemporal organization of the rhizosphere – a key to rhizosphere functions”, funded by the German Research Foundation, a hierarchical sampling approach for the

lab and the field scale was developed. For the large scale (7 cm \AA) X-ray CT scanning provides temporal and spatially resolved data on root architecture, root age, diffusion lengths/travel distances and soil structure development which integrates information from in situ soil solution samplers, spatially informed sampling of the microbiome, the root gene expression and exudation as well as enzyme activity. At the small scale (1.6 cm \AA subsamples) X-ray CT scanning enables registration of 2D chemical information from different microscopy and micro-spectroscopy methods, and potentially also information on distribution of microbes, back into the 3D context. By merging the scales root age information is available for both approaches and can be used to explain root-age specific uptake and release patterns. Drivers for pattern formation are corn genotypes differing in root hair formation (WT, *rth3*) and two different soil textures (sand, loam) differing in transport properties. First results for the central experimental platforms established at field and lab scale will be presented.

11:00 - 11:15

ROOTS CHANGE THE PORE STRUCTURE ONLY IF THEY HAVE TO – DEVELOPMENT OF BIOPORES AND COMPACTION AROUND ROOTS.

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The interaction of plant roots with weathered mineral material shapes unique structures depending on the parental material and the site specific climatic conditions. Degraded soil structure on the other hand can lead to limited root growth and therefore crop yields.

Here we explored these interactions within a chronosequence in the Rhenish lignite mining area in Germany. We were able to investigate the development of biopores over time (24 years) by separating them from other structural pores based on shape.

For this, X-ray CT was used for scanning around 300 soil columns (3 cm diameter) covering top (0-20 cm) and subsoil (40-60 cm) from different sites ranging in age from 0 to 24 years. By destructive sampling and analysing with WinRHIZO, information about root length densities could be derived and correlated with pore structure.

An increase in biopore density throughout year zero to year 12, in particular in 40-60 cm soil depth could be observed. The biopore length density of approximately 16 cm / cm³ obtained in year 12 was similar to the one measured in year 24, suggesting that equilibrium was reached. Only about 10% of these biopores were filled with roots.

In the second part of this study, the CT derived information (visible porosity, gray value) was used to calculate the density around biopores. Whether biopores were surrounded by soil with higher or lower porosity compared to the bulk soil was dependent on plot age and depth. In plots with low macroporosity a significant compaction around biopores was observed. A greenhouse experiment with maize, growing for 20 days in three different bulk densities (1.3, 1.45 and 1.6 g cm⁻³), corresponding to different macroporosity, corroborated a clear interaction between macroporosity and root induced compaction.

The current study is part of the DFG-Project Soil Structure (AOBJ: 628683).

11:15 - 11:30

RHIZOSPHERE MODELLING REVEALS THE SPATIOTEMPORAL DISTRIBUTION OF DAIDZEIN, WHICH ALTERS BACTERIAL COMMUNITIES IN THE RHIZOSPHERE

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Rhizosphere is pivotal in both nutrient uptake and interaction with diverse range of microbes in the soil. Various researches provided evidences that plant metabolites secreted from roots provoke the changes of rhizosphere microbial communities and mediate interactions of both symbiotic and pathogenic, suggesting the importance of these metabolites to promote growth and health of plants. Despite the importance of the root-secreted metabolites in the rhizosphere, little is known on the distribution and fate in the rhizosphere. We have studied the secretion of daidzein, an isoflavone, from soybean roots and found that secretion is higher during the vegetative stages than during the reproductive stages both in hydroponic culture and field condition. In this study, we used daidzein as a model to study the dynamics in the rhizosphere. Rhizosphere modelling was performed with advection dispersion equation, using the parameters such as daidzein distribution coefficient and degradation coefficients in soybean field soil. Simulation showed that daidzein distribution from roots was limited within 2 mm. The distribution of daidzein in sands was simulated to be further beyond 10 mm. Simulation of daidzein in both field soil (lowland gray soil) and sands was then validated in rhizobox. In order to clarify the influence of daidzein on rhizosphere bacterial communities, we made an artificial rhizosphere environment *in vitro*, and maintained soils with various concentration of daidzein. The bacterial communities were altered depending on the concentration of daidzein, and it was shown that daidzein at the concentration observed in the rhizosphere provoked the significant changes of bacterial communities within 2 weeks. Bacteria belonging to the Family increased by daidzein were isolated from soybean rhizosphere, and functionally characterized. In this presentation novel plant-microbe interaction mediated by daidzein will be discussed.

11:30 - 11:45

HYDRAULIC BRIDGES AT THE ROOT-SOIL INTERFACE: INSIGHTS FROM HIGH-RESOLUTION SYNCHROTRON X-RAY CT

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Although 40% of total terrestrial precipitation transits the rhizosphere, there is still substantive lack of understanding of the rhizosphere biophysical properties and their impact on root water uptake. Our hypothesis is that roots are capable of altering the biophysical properties of the rhizosphere hereby facilitating root water uptake. In particular, we expect that root hairs and mucilage maintain the hydraulic contact between roots and soil at low water potentials. We have recently shown that root hairs facilitate root water uptake in dry soils,

especially at high transpiration rate. Our explanation was that root hairs extend the effective root radius decreasing the flow velocity at the root surface and hence the drop in matric potential across the rhizosphere. To test this hypothesis, we used synchrotron X-ray CT to image the distribution of root hairs in soils and quantify the contact area between hairs and the soil matrix. The experiments were conducted with two maize genotypes (with and without root hairs) grown in two soil textures (loam vs sand). We found that root hairs significantly increased the root-soil contact area and bridged the air-filled pores between the root epidermis and the soil matrix. Interestingly, we also observed morphologically distinct filaments emerging from the root epidermis and extending across the air-filled pore space. These filaments could be fungal hyphae or mucilage threads similar to those that we have previously seen in soils mixed with mucilage. We have then segmented the root hairs, the filaments and the associated soil pores and used the segmented geometry to develop a simplified model of root water uptake including the effect of root hairs and filaments at the pore scale. In summary, high-resolution imaging of the root-soil interface revealed the presence of elements (hairs and mucilage or fungal hyphae) maintaining the roots in contact to the soil matrix.

11:45 - 12:00

CONTRASTING IMPACTS OF RHIZODEPOSITS ON THE PHYSICAL FORMATION AND FUNCTIONING OF THE RHIZOSPHERE

PD Hallett¹, M Marin¹, M Naveed^{1,2}, E Oleghe^{1,3}, TS George⁴, LK Brown⁴, AG Bengough^{4,5}, T Roose⁶, L Cooper⁶, N Koebernick⁶, S Ruiz⁶

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Not all rhizodeposits are created equal. We observed that maize rhizodeposits contain sticky hydrogels that trap water and aggregate soils, whereas barley rhizodeposits disperse soil particles and act as a surfactant. These effects, however, are short-lived as the hydrogel and aggregating properties of rhizodeposits diminish for maize and increase for barley during microbial decomposition. A series of experiments will be presented, starting with direct physical characterisation of the surface tension and viscosity of rhizodeposits. Maize rhizodeposits are about 10 x more viscous than barley, with maize and barley decreasing surface tension by more than 30% and 40%, respectively. These properties are reflected in water retention. Soil amended with maize rhizodeposits retained more water and had accentuated hysteresis between drying and wetting limbs, whereas barley rhizodeposits decreased water retention and hysteresis. Rheological tests found that barley rhizodeposits initially weakened soil by 50%, suggesting dispersion that could liberate nutrients and ease root penetration. Maize rhizodeposits doubled the strength of soil, so act as a stabiliser. However, after microbial decomposition, the strength of both barley or maize rhizodeposit amended soil were the same as unamended soils. The differing physical behaviour between maize and barley rhizodeposits is reflected in their chemistry. Maize rhizodeposits contain a greater proportion of polysaccharide derived sugars, whereas barley

rhizodeposits contain a greater proportion of organic acids. Plants appear to have evolved different mechanisms to interact with soil physical behaviour, affecting how they influence water retention and capture, aggregation and dispersion to access soil nutrients.

This research has extended to consider a greater range of environmental variables, such as the interaction between rhizodeposits and organic residues in soil, and effects of physical weathering. Smaller-scale testing approaches have also been developed to allow for direct measurements at the root-soil interface. These findings link directly with image-based modelling and analysis of rhizosphere formation and functioning.

12:00 - 12:15

SIMULATING AND CHARACTERIZING SPATIO-TEMPORAL PATTERNS OF RHIZODEPOSITS-THE DEVELOPMENT OF THE RHIZOSPHERE IN 3D

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The rhizosphere refers to the soil area that is directly and actively influenced by root exudation and secretions. We developed a 3D mathematical model based on analytical solutions that quantifies the contribution of release patterns, root growth dynamics, solute transport and degradation to rhizodeposit distribution, both along a single growing root and within the rooting zone of a growing plant.

The model is based on analytical solutions of the 3D diffusion-reaction equation coupled to a root growth model. The application of the model is illustrated for two kinds of rhizodeposits (citrate and mucilage) with respective diffusion, sorption and degradation coefficients which were simulated for two plant species differing in root architecture (*Vicia faba* and *Zea mays*). We defined measures that characterized the spatio-temporal distribution of the rhizodeposit such as the dilution index, hotspot volumes and hot moment times and used them to evaluate the impact of root architecture and rhizodeposit properties (release and decay rates, diffusion, sorption).

Each root develops a steady state rhizodeposit distribution with respect to the moving root tip. Our simulations show that the radial extent is larger for citrate than for mucilage and thus the change of overlap volume of the rhizodeposit between different roots is higher in the case of citrate. This is supported by a larger dilution index for citrate than for mucilage.

This is the first model to quantify the spatio-temporal distribution of rhizodeposits associated with 3D root growth, improving our understanding of joint impact of spatio-temporal distribution of rhizodeposits and the development of root system on rhizosphere development.

12:15 - 12:30

RESPONSE OF SOIL MICROBIAL COMMUNITY TO PHOTOSYNTHETIC PRODUCT SECRETION ACTIVITY OF PLANT ROOTS INDICATED BY RHIZOSPHERE LIVE IMAGING METHOD

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The quality and quantity of photosynthetic products secreted from plant roots are vary depending on root parts and states, and changes in the surrounding environment. Depending on the difference in the secretion activity of the roots, it is pointed out that the structure and function of the rhizosphere microbial community are highly dynamic both spatially and temporarily. To enable measurement of the secretion activity at each position of the roots at a specific time point which is difficult hitherto to be measured, we used ¹¹C₂ pulse labeling and a positron-emitting tracer imaging system to visualize the translocation of photosynthetic products to the root system and the distribution of ¹¹C-labeled products secreted into the rhizosphere soil.

The ¹¹C₂ tracer gas was supplied to the above-ground part of the white lupin cultivated in a rhizobox dividing the roots and the soil by a nylon mesh. The rhizobox was disassembled at 80 minutes after the addition of the tracer, and the distribution of the ¹¹C-labeled photosynthetic products secreted into the rhizosphere soil was evaluated. After that, the soil was divided into lattice-shaped sections at intervals of 2 cm, and the organic and inorganic substances contained in soluble fractions and microbial community were analyzed.

SESSION 3: ROOT EXUDATES

(GALLERY C)

Session Chairs: Melissa Arcand & Eduardo Mitter

10:30 - 11:00

SAMPLING ROOT EXUDATES – MISSION IMPOSSIBLE?

E Oburger¹, DL Jones²

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Accurate information about the quantity, quality and spatiotemporal dynamics of metabolite release from plant roots is vital to understanding the functional significance of root exudates in biogeochemical processes occurring at the root-microbe-soil-interface. Significant progress in analytical techniques nowadays allows us to gain a much better picture of the rich diversity of compounds that are present in root exudates, but ultimately the choice of exudation sampling strategy will determine the ecological significance of obtained exudation results. Unfortunately, in the past, little consideration has been given to the experimental strategy used to sample root exudates. To date, our knowledge on root exudation is mainly based on plants grown and sampled in nutrient solution culture (hydroponics). Despite the operational benefit of hydroponic systems, the question remains as to how ecologically relevant exudation results obtained under these artificial conditions

are compared to soil environments, particularly in the context of exudate driven rhizosphere processes. While a perfect method for sampling root exudates does not exist, soil based approaches, if appropriately applied and interpreted, may still provide more realistic insights into exudation dynamics in natural soil environments. Based on a recently published literature review, we aim to provide an overview of different root exudation sampling approaches and their advantages and limitations to support the selection of the most suitable experimental procedure for any specific research question. Furthermore, we address critical methodological aspects that need to be considered in the choice of experimental approach, like growth and sampling medium (soil, hydroponic), sterility, sampling location (whole root system, individual root segments) as well as plant age, daytime, re-uptake of metabolites affecting duration and timing of the sampling event and data presentation.

11:00 - 11:15

DIGGING INTO SOIL CHEMISTRY – METABOLOMICS ANALYSIS OF ROOT EXUDATES AND RHIZOSPHERE METABOLITES

A Weinhold^{1,2}, GA Garcia De Leo^{1,2}, C Ristok^{1,2}, K Potthast³, A Tischer³, B Michalzik^{1,3}, NM van Dam^{1,2,4}

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The rhizosphere is a place where many interactions between plants and their environment take place. This includes interactions with other plants, pathogens, symbionts or herbivores as well as organic and inorganic forms of nutrients. Many of these interactions are mediated by chemical compounds. Rhizosphere chemicals, or metabolites, can be of different origins. They can be produced by soil biota, exuded by plant roots or leached from litter. Root exudates and litter leachates, in turn, can affect soil biota, and alter the composition of microbial communities. The exact nature of the compounds involved in belowground signaling is mostly unknown. Untargeted metabolomics analyses provide the understanding how plant metabolites are shaping interactions in the soil. Here, we show how metabolomics can help to elucidate the chemicals mediating interactions in the rhizosphere. We analyzed soil metabolites from two independent experiments. Within the first experiment, we analyzed root exudates of four tree species grown in a biodiversity-ecosystem functioning experiment. We conducted the second experiment to test the effect of herbivory (aphid infestation) on litter leachate as well as on the chemical composition of different soil layers originated from a plant-soil mesocosm system. We analyzed all samples by liquid chromatography – mass spectrometry. Our Results show: i) that plant diversity affects the chemical composition of root exudates (experiment 1), and ii) that aboveground herbivory modulates the chemical composition of leaf litter leachates, which percolates through the soil (experiment 2).

11:15 - 11:30

QUANTIFYING CITRATE-ENHANCED PHOSPHATE UPTAKE BY ROOTS

D McKay Fletcher¹, R Shaw², A. Sánchez-Rodríguez^{2,3}, A van Veelen¹, D Jones², T Roose¹

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Citrate root exudation is thought to be important for root phosphorus absorption in low phosphorus soils. However, quantifying the dynamic effect of citrate-enhanced phosphate desorption in soil using conventional experimental methods is difficult as these methods disrupt the soil and cannot offer good time resolved results. Mathematical modelling is often used to predict the effect of enhanced desorption, however, many important parameters in the past models are unknown and/or often speculative. In this work, we quantify citrate-enhanced phosphate desorption and its importance for root uptake by combining modelling with experiments conducted using microdialysis probes.

Microdialysis offers a minimally-invasive approach to sample phosphate quantities in soil, while simultaneously exuding known amounts of citrate into the soil. Inferring direct measurements by using microdialysis in soil is difficult as chemical properties of the soil and perfusate can affect the osmosis rates. Microdialysis probes were placed in soil perfused with citrate to measure available phosphate with high temporal frequency. To aid interpretation of the microdialysis results, a mathematical model describing the experiments was fit to the experimental data. In the fitting, the rate at which citrate mobilises adsorbed phosphate was estimated to be m^3 of soil solid $s^{-1} \mu mol^{-1}$ of citrate. This enabled us to parameterise a model of an exuding root, which was used to quantify the importance of citrate-enhanced phosphate desorption for plant uptake. As the citrate-enhanced desorption rate was small, a single root exuding citrate at a typical rate did not contribute significantly to phosphate uptake. However, densely packed roots could combine and exude enough citrate into the soil to greatly increase phosphate mobility and root uptake. Furthermore, the model predicted that citrate enhanced desorption was more important for strongly adsorbed phosphate.

11:30 - 11:45

ORGANIC ACIDS AND PHOSPHATASE ENZYMES DETERMINE PHOSPHORUS AVAILABILITY IN THE RHIZOSPHERE OF DIFFERENT PLANT SPECIES.

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¹*Soil Science, Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, Canterbury, New Zealand*

Phosphorus is a key nutrient for crop production, and rhizosphere processes play a critical role in P acquisition and availability. The objective of this study was to investigate the effect of phosphorus addition on rhizosphere processes and phosphorus mobilization. Therefore, a glasshouse pot experiment was carried out and involved growing different plant species (*Lupinus albus*, *Trifolium Repens* L, *Lolium perenne*, and *Triticum aestivum* L.) in a low fertility grassland soil (4 ppm Olsen P) with or without phosphorus addition for 8 weeks. At the end of the

experiment plant root and shoot biomass, phosphorus content together with organic acids, phosphatase activity and microbial biomass were measured. Soil phosphorus depletion was also calculated by difference between phosphorus fractions in the original soil (amended and no amended) and the rhizosphere soil after plant growth. As expected phosphorus addition increased total phosphorus uptake for all plant species. Phosphorus addition increased phosphorus microbial biomass with a significant difference in white clover and ryegrass compared with the control. Acid and alkaline phosphatase activity were not affected by phosphorus availability, however white lupine had the highest alkaline phosphatase activity (2.06 $\mu\text{mol/g/h}$). The total concentration of organic acids in the rhizosphere of white lupine and white clover increased by 8,5 folds and 11 folds after phosphorus addition, respectively. After phosphorus addition, all plants depleted inorganic phosphorus fractions especially white lupin and white clover, whereas wheat, white lupin, and white clover depleted organic phosphorus fractions. Regression analysis revealed that phosphorus depletion in legumes was related to organic acids exudation in the rhizosphere, while phosphatase activity was significantly correlated to P mobilisation in wheat rhizosphere. The results from our study emphasized that physiological mechanisms are important to phosphorus acquisition and that different plant species rely on divergent processes to enhance their phosphorus uptake.

11:45 - 12:00

HOW RICE ROOT EXUDATES SHAPE THE NITRIFICATION PROCESS?

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Microbial nitrification in soils is a major contributor to nitrogen (N) loss in agricultural systems. Some plants can secrete root exudates that act as biological nitrification inhibitors (BNIs), and a small number of BNIs have been identified and characterized. However, virtually no research has focused on the important food crop, rice (*Oryza sativa*). Here, 19 rice varieties were explored for BNI potential on the key nitrifying bacterium *Nitrosomonas europaea*. Exudates from both indica and japonica genotypes were found to possess strong BNI potential. A new nitrification inhibitor, 1,9-decanediol, was identified, shown to block the ammonia monooxygenase (AMO) pathway of ammonia oxidation. Plant N-use efficiency (NUE) was determined using a ¹⁵N-labeling method. Correlation analyses indicated that both BNI abilities and 1,9-decanediol amounts of root exudates were positively correlated with plant ammonium-use efficiency and ammonium preference. The release of 1,9-decanediol is enhanced by low to moderate concentrations of NH_4^+ ($\leq 1.0\text{mM}$), low pH, aeration of the rhizosphere, and nitrifying bacteria. These findings provide important new insights into the plant–bacterial interactions involved in the soil N cycle, and improve our understanding of the BNI capacity of rice in the context of NUE.

12:00 - 12:15

WHO'S YOUR NEIGHBOR? PLANTS NEIGHBORS INDUCE SPECIES-SPECIFIC CHANGES IN A FOCAL PLANTS' ROOT EXUDATION AND RHIZOSPHERE COMMUNITY COMPOSITION

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Plants simultaneously interact with each other and soil microorganisms, yet most studies independently focus on either plant-plant or plant-microbe interactions. For instance, plant soil feedback experiments use a soil-conditioning technique that predicts the effects of current plant-microbe interactions on future plant populations; these experiments fail to capture how active interactions between plants may alter microbial communities. Plant interactions, including competition for resources and chemical signaling, have been shown to alter plant morphology, metabolic profiles, and fitness, but little is known about how these neighbor-induced changes alter a plant's selection of its microbial community. Plants use root exudates, complex chemical cocktails, to alter their microbial communities, access nutrients, and signal other plants. Therefore, we predict that neighbor-induced changes in root exudates may be one mechanism by which neighboring plants alter each other's microbial communities. We tested this in a greenhouse experiment in which a focal C4 grass, Switchgrass, (*Panicum virgatum*) was grown alone, neighboring a conspecific, or one of three native prairie species— *Andropogon gerardii* (C4 grass), *Koeleria cristata* (C3 grass), or *Rudbeckia hirta* (forb). Using untargeted metabolomics and 16S amplicon sequencing, we found that neighbor identity explains 54% of the variation in focal plant root exudate composition and 20% of the variation in rhizospheric bacterial community composition. The exudate and bacterial compositions were significantly correlated, suggesting that neighbor-induced shifts in the focal plant's exudates drive shifts in its bacterial community. These results demonstrate that active plant-plant interactions can alter microbial communities. Future studies should consider that plant-microbial interactions studied in isolation from neighboring plants will likely change when placed in a neighborhood context and, further, that neighbor-induced microbial changes can alter plant-plant interactions beyond the legacy effects predicted by plant soil feedback experiments.

12:15 - 12:30

COMPARATIVE ANALYSIS OF ROOT EXUDATES AND BACTERIAL DIVERSITY IN AEROPONICS AND SOIL

B Kim¹, M Bentlage¹, B Thiombiano¹, H Bouwmeester¹, **A Zancarini**¹

¹*Plant Hormone Biology group, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands*

In the context of highly pressured agricultural production using low levels of inputs, cropping strategies could take advantage of plant-microbiome interactions that can improve both plant growth and health. To do so, a

better comprehension of the mechanisms that drives these interactions, such as the exudation of signalling molecules by plants, is needed. However, collection and analysis of the root exudates show significant technical challenges. Indeed, root exudates are mostly studied in artificial conditions, such as in hydroponics, while they are affected by microbial presence and soil properties.

Here, we compared root exudates and bacterial diversity for two tomato genotypes under three different growing conditions (*i.e.* in aeroponics and two natural soils). We analysed root exudates using a metabolomics approach and we assessed bacterial diversity and composition using a metabarcoding approach (16S amplicon sequencing). Using multivariate analysis, we observed a significant, mainly quantitative, effect of the growing conditions on the root exudate composition. The bacterial diversity decreased from bulk soil and the rhizosphere to the root itself. In addition, even at the phylum level, the belowground bacterial community composition was significantly different according to the compartment and the soil. However, the root bacterial diversity and composition of the two tomato genotypes were similar in aeroponics and in the two soils. This suggests that the root microbiome is not so much affected by horizontal transfer of microbes from the soil. Thus, within this study, we developed a method to analyse root exudates in soil. Further advanced statistical analyses will be used to link the root exudate profile to the microbiome composition in order to find the molecular mechanisms involved in microbiome selection by plants.

12:30 - 14:00

LUNCH

(CENTENNIAL HALL)

14:00 - 15:00

KEYNOTE ADDRESS - CORNE PIETERSE

(SALON B+C)

THE SOIL-BORNE SUPREMACY: ROOT MICROBIOME AND PLANT IMMUNITY

C. Pieterse¹, G. Stringlis¹, K. Yu¹, G. Vismans¹, Y. Song¹, E. Verbon¹, P. Trapet¹, R. de Jonge¹, R. Berendsen¹, P. Bakker¹

¹*Utrecht University, Utrecht, Netherlands*

Plant roots nurture a large community of microbiota that provide them with essential services, such as enhanced nutrient uptake, growth promotion, and protection against pathogens. Our research is focused on understanding plant-beneficial functions encoded by the root microbiome and the role of plant genes facilitating these functions. We demonstrated that infection of *Arabidopsis* leaves with the downy mildew pathogen *Hyaloperonospora arabidopsis* results in the build-up of a microbial community in the rhizosphere that confers enhanced protection against this pathogen in a second population of plants growing in the same soil (*ISME J* 12:1496–1507; *Cell* 172:1178-1180). Root microbiome analysis revealed a consortium of synergistic *Stenotrophomonas*, *Microbacterium*, and *Xanthomonas* strains that collectively induced systemic resistance (ISR) against the pathogen that initiated their recruitment in the rhizosphere. Previously, the root-specific transcription factor MYB72 emerged as an important regulator of ISR (*Annu. Rev. Phytopathol.* 52:347–75; *Annu. Rev. Phytopathol.* 55:355-75). Preliminary findings suggest that MYB72 is also important for the ISR-eliciting soil-borne legacy that develops in the rhizosphere of downy mildew-infected plants. MYB72 is specifically activated in epidermal root cells in response to ISR-inducing rhizobacteria, but also in response to iron starvation (*Plant J.* 84:308-22). Besides being essential for ISR, MYB72 regulates the biosynthesis and excretion of iron-mobilizing fluorescent phenolic compounds into the rhizosphere (*New Phytol.* 204:368-79).

Metabolite fingerprinting revealed the antimicrobial coumarin scopoletin as a dominant MYB72-dependent metabolite that is excreted into the rhizosphere, where it aids in iron uptake (*PNAS* 115:5213-5222). ISR-eliciting rhizobacteria, such as *Pseudomonas simiae* WCS417 and *Pseudomonas capeferrum* WCS358 are insensitive to the antimicrobial activity of scopoletin. Microbiome analysis of wildtype and scopoletin-deficient mutant plants demonstrated that plants and PGPR join forces to trigger MYB72-dependent coumarin excretion, resulting in improved niche establishment for the microbial partner and enhanced iron uptake, growth, and immunity benefits for the host plant.

15:15 – 16:45 PARALLEL SESSIONS

SESSION 4: THE ROOT MICROBIOME

(GALLERY A)

Session Chairs: Davide Francioli & Sue Grayston

15:15 - 15:45

HOW BIOTIC INTERACTIONS SHAPE THE MOVEMENT OF FRESH AND DECAYING ROOT CARBON INTO SOIL

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While the soil surrounding plant roots may comprise only 1-2% of the total soil volume, this zone can provide 30-40% of the total soil organic carbon input and is a nexus for microbial C transformations. Stimulated by exudates and decaying roots, rhizosphere organisms (bacteria, archaea, fungi, fauna, and viruses) interact to move carbon from root tissue to surrounding soil, and ultimately regulate how soil C is stabilized. While soil food web concepts are well established, a quantitative and mechanistic understanding of how networks of organisms control soil organic matter dynamics is only recently emerging. In our experiments, we use ¹³CO₂-grown *Avena fatua* plants (common in Mediterranean grasslands) to introduce carbon into soil, and isotope tracing (stable isotope probing-metagenomics, NanoSIMS imaging, soil density fractionation) to delineate the complex web of interactions. Microbial densities and activities were often ten times higher in the rhizosphere compared to surrounding bulk soil. As roots grew and matured, the distinction between rhizosphere and background bulk community composition became more pronounced and microbial gene expression evolved rapidly; carbohydrate depolymerization genes were upregulated near living roots, and as the rhizosphere aged, decomposition functions became subdivided amongst an increasing number of microbial guilds. Overall, microbial functional capacity was the best predictor of root decomposition rates. We also found that populations of phages that incorporated root-derived ¹³C were distinct from non-labelled phages, indicating a cohort that must specialize by targeting bacteria that feed on root carbon. We use gradient centrifugation and size selection to physically separate nematodes and protists from soil, and found sequenced libraries yielded

50-70% faunal sequences. Nematodes and protists were both more abundant in rhizosphere than bulk soil and predicted functional roles of these guilds suggest direct root feeding and bacterial and fungal predation.

15:45 - 16:00

CLOSE-UP ON INFORMATIONAL WAR BETWEEN GRAM OPPOSITE RHIZOBACTERIA

A. Chane¹, C. Barbey^{1,2}, Y. Bourigault¹, S. Rodrigues¹, M. Bouteiller¹, A. Merieau¹, Y. Konto-Ghiorghi¹, A. Beury-Cirou², M. Feuilloley¹, V. Gobert², M. Patek³, X. Latour¹

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³*Institute of Microbiology of the CAS, Praha, Czech Republic*

The rhizosphere hosts microbes that establish communication networks as well as others that disrupt them. These cell-to-cell communication systems are based on both the synthesis and perception of diffusible signaling molecules, the best known of which belong to the *N*-acyl-homoserine lactone family. When a threshold population density is reached (i.e. quorum), the corresponding threshold signal concentration can be sensed, triggering the expression of virulence and the onset of disease symptoms. Among rhizobacteria, some Gram-positive Actinobacteria have the ability to sensor the signaling molecules produced by soft-rot Gram-negative phytopathogens in order to quench their quorum-sensing communication and to impair their social and virulent behavior.

We developed dual-color reporter strains suited for monitoring quorum-sensing and quorum-quenching activities, respectively. A constitutively expressed cyan (pathogen) or red (biocontrol agent) fluorescent protein served as a cell tag for plant colonization. Besides, an inducible expression reporter system based on the green fluorescent protein gene enabled the simultaneous recording of signaling molecule production (by pathogen), detection and/or degradation (by biocontrol agent). The dual-colored pathogen (blue when inactive and green during quorum-sensing) and the biocontrol strain (red when inactive and yellow during quenching) were introduced alone or together in the *Solanum tuberosum*-pathosystem.

At cellular quorum, confocal microscopy images revealed the colonization and damage caused by the soft-rot bacteria, the latter being associated with a strong pectobacterial quorum-sensing activity, especially at the plant cell walls. When the biocontrol agent and pathogen are co-inoculated, the quorum-sensing activity is jammed by a subtle and concomitant quorum-quenching response both at the single-cell and microcolony levels, leading to a powerful plant protection. The generated biosensors appear to be promising and complementary tools useful for molecular and cellular studies of bacterial metabolism, communication and interference.

16:00 - 16:15

SHOTGUN METAGENOMICS SEQUENCING OF THE RHIZOSPHERE MICROBIOTA ASSOCIATED TO SEVEN ECOTYPES OF *MEDICAGO TRUNCATULA*.

J Cremaschi¹, O Rué², F Deau¹, P Lemanceau¹, V Loux², S Mondy¹, **B Pivato**¹

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Healthy plants host a remarkable diversity of microorganisms known as plant microbiota, which provide host services such as pathogen protection and nutrient acquisition. Thus, plant microbiota emerges as a trait that extends the capacity of plants to adapt to their environment. So far, microbial community profiling has mostly allowed the description of the phylogenetic structure of plant microbiota, whereas functional insights were mostly obtained from experiments using model strains. Thus, the plant impact on the microbial functional genes pool in the rhizosphere remains largely unknown.

The goals of the study were to (i) compare the functional genes pool of the rhizosphere microbiota of seven ecotypes of *Medicago truncatula*, and (ii) identify the functional genes allowing the differentiation of the microbiota upon the plant ecotypes.

Forty-two samples were analysed for their phylogenetic (454 pyrosequencing of 16S rRNA) and functional genes (2x125 bp shotgun sequencing, Illumina HiSeq) diversity. Fifty-five millions scaffolds were *de novo* assembled after removing reads mapped on *M. truncatula* genome. We used Prodigal as gene prediction from mRNA scaffolds and Kaiju for taxonomic classification of rRNA scaffolds. The three millions predicted mRNA (≥ 800 bp length) were filtered to remove scaffolds that carried transposable elements or came from organelles or *Sinorhizobium sp.* symbionts. A selected dataset of 6074 scaffolds ≥ 10 kb was used for statistical analyses.

Results indicated that (i) the genetic structure of bacterial communities differed according to the plant ecotype, and (ii) the curation of the shotgun metagenomic dataset is necessary to have a better discrimination of the different rhizosphere microbiota. Furthermore, the membrane receptor TonB protein appeared to explain differences in the structure of the rhizosphere microbiota associated with the different ecotypes. Indeed, these proteins bind and transport ferric chelates made with siderophores, as well as various plant carbohydrates, and thus to mediate plant-microbe interactions.

16:15 - 16:30

INTENDED AND UNINTENDED CONSEQUENCES OF PLANTED FOREST MANAGEMENT FOR MICROBIAL RHIZOBIOME PROPERTIES

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The importance of mycorrhizae to forest productivity has long been recognised, but the contribution of the wider microbial rhizobiome to plantation performance is less well characterised. Globally, forest management is intensifying to meet increasing demand, and it is important to determine if this intensification is producing unintentional negative effects for critical rhizobiome functions. This concern, combined with a growing

appreciation for the potential to enhance forest productivity by “encouraging” beneficial microbial activity, led to the establishment of several trials incorporating assessments of rhizobiome structure and function across the New Zealand planted forest sector. Here we discuss some of our key findings.

Investigations of chemical use in tree nursery environments have determined that radiata pine mycorrhizae are highly sensitive to chemical management. Fungicide use to control pathogens suppressed the most beneficial mycorrhizal species, generating negative legacy effects for tree growth that persisted after planting in the forest, reducing productivity by 8% after six years. Plant growth promoting rhizobacteria associated with young radiata pine responded positively to fertiliser use, increasing the abundance of bacterial genes associated with plant stress tolerance, and reducing the negative effects of a 6-week drought by 48%.

Within the plantation, application of a novel fertiliser enhanced the abundance of beneficial rhizobacterial genes, linked to gains of 7% in growth and 17% in disease tolerance. The selection of radiata pine genotype had a massive impact on the abundance of plant growth promoting genes in the rhizobiome, and positively correlated to the disease tolerance of those genotypes. However, various practices employed to increase yield, such as increased organic matter removal at harvest, were found to persistently reduce beneficial microbial activity.

Overall, there is considerable opportunity to persistently enhance forest productivity by intentionally managing for positive rhizobiome activity, but this sensitivity to management also enables unintended negative response to manifest.

16:30 - 16:45

A SYNTHETIC COMMUNITY APPROACH TO UNDERSTANDING THE POPLAR MICROBIOME

D Carper¹, D Weston¹, S Jawdy¹, L Burdick¹, D Pelletier¹

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Field grown *Populus* harbor a diverse consortium of microbes. To gain insight into the functional role of these microbial members on the *Populus* host, we isolated over 3,200 strain bacterial strains using a direct plating approach. Our prior studies used this collection with individual strain and very reduced community (< 10 members) assays to evaluate questions pertaining to plant – microbe interactions. However, such studies have little relevance to boarder community-based questions, such as the rules governing community assembly and the relationship between diversity and function. To address this limitation, we created a synthetic community approach that distinguishes the genetic diversity of genome sequenced strains using Illumina 16S rRNA sequencing. We are now using this approach to inoculate germ-free *Populus trichocarpa* with relatively large bacterial communities to determine how host plant genetics and nutrients interact to shape the associated community, and in turn, how that specific community influences plant nutrient status. To create the synthetic community, all 16S rRNA sequences from the sequenced bacterial strains within the culture collection were extracted, trimmed to the specific Illumina region and aligned using a custom computational workflow. Our workflow was scripted to design a community that had at least three nucleotide differences within the 16S rRNA region. This resulted in a community consisting of 154 members that spans substantial genetic diversity including 4 phyla, 9 classes, 12 orders, 32 families and 77 genera. Many strains contain potentially beneficial functions for the host including nitrogenase activity, indole-3-acetic acid production and anti-microbial

production. Initial experiments with reduced ten-member communities have shown that community assembly is surprisingly reproducibly, and that specific community members impact host plant nitrogen status. Our current experiments are investigating whether these findings are observed within much larger communities.

SESSION 5: SPATIO-TEMPORAL ORGANIZATION IN THE RHIZOSPHERE

(GALLERY B)

Chairs: Doris Vetterlein

15:15 - 15:45

INSIGHTS INTO THE SPATIAL ORGANIZATION OF RICE-ASSOCIATED DIAZOTROPHS AND THEIR NITROGEN FIXATION ACTIVITY

H Schmidt¹

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Rice is one of the world's most important crop plants. The production of wetland rice demands high nitrogen inputs which are commonly provided by hazardous and costly industrial fertilizers. For decades, it has been known that Biological Nitrogen Fixation through non-symbiotic diazotrophs can alleviate nitrogen shortage in rice cultivation in a more eco-friendly manner. However, major questions regarding the identity of active microbial players, their diversity in various soil and root compartments, as well as the transfer of nitrogen to rice plants remain unanswered.

With a focus on the spatial organization of diazotrophic communities in root-soil interfaces I will present novel data obtained through mesocosm and gnotobiotic experiments and analyzed via amplicon sequencing, stable isotope tracer assays, spatial statistics, and single-cell imaging. Our data shows that diazotrophic communities were more strongly influenced by soil type than by rice genotype and that communities diversified into microenvironments according to their lifestyles (anaerobic in bulk soil vs aerobic in root). Bulk and single-cell analyses of isotopic enrichment indicated that both oxic and anoxic plant-soil microenvironments harbored active nitrogen fixing bacteria that could potentially contribute nitrogen to the plant. Root-associated cells showed significant spatial clustering and tended to be located around root cell borders which could present potential hotspots of nitrogen fixation. A newly developed stable-isotope imaging strategy confirmed the *in situ* nitrogen fixation activity of single diazotrophs situated in such hotspots on rice rhizoplanes.

Overall, I will provide novel insights into Biological Nitrogen Fixation in wetland rice cultivation, ranging from the microbial community scale down to single diazotrophic cells.

15:45 - 16:00

SPATIAL EVALUATION OF CARBON FLOW AND MICROBIAL FUNCTION THROUGH THE ROOT-RHIZOSPHERE-SOIL CONTINUUM

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We are testing the central hypothesis that spatially focused regions within the rhizosphere funnel a disproportionately high amount of nutrients to a plant. We further hypothesize that the localization of these active areas is controlled by heterogeneity in soil geochemistry, distribution of plant carbon input, and the distribution of microbial populations and function. We are using multi-pronged, spatially-resolved analyses to interrogate the carbon status, microbial activity, and nutrient content of switchgrass microcosms constructed with natural soil (Kellogg Biological Station, Hickory Corners, Michigan, USA). We adapted laser ablation-isotope ratio mass spectrometry for analysis of rhizosphere samples and used the approach coupled with a ¹³C tracer to track variable rates of photosynthate flow into different roots and the rhizosphere. We are also exploring the use of spectroscopy-based ¹³C quantification to improve measurement sensitivity and spatial resolution. We are developing two methods to evaluate the microbial components and activity within the system: 1) spatially resolved proteomics assays and 2) selective activity-based staining of specific enzymatic functions (e.g., phosphatase activity). Our technique involves transferring mobile phase proteins onto a membrane while maintaining their spatial distribution in the soil. This technique is non-destructive to the host plant and enables timeseries analysis of the microbial community. We developed a laser-induced breakdown spectroscopy technique to enable mapping of macro- and micro-nutrients in the soil surrounding roots and demonstrated its ability to identify specific elemental foci that may support hotspots of microbial activity. Finally, we are developing quantitative image analysis packages to identify relevant geochemical, microbial activity, and carbon gradients operating through the root-rhizosphere-soil continuum and to subsequently elucidate covariance of these patterns that may contribute to observed biogeochemical processes. Together, integrating these data types will reveal how soil geochemical microenvironments and microbial activity relate to the distribution of fresh photosynthate provided by the plant.

16:00 - 16:15

BENCHMARKING MODELS OF ROOT ARCHITECTURE AND FUNCTION

A Schnepf¹

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3D models of root growth, architecture and function are evolving as they become important tools that aid the design of agricultural management schemes and the selection of beneficial root traits. While benchmarking is common for water and solute transport models in soil, 3D root-soil interaction models have never been systematically analysed. Several interacting processes might induce disagreement between models: root growth, sink term definitions of root water and solute uptake and representation of the rhizosphere. The extent

of discrepancies is currently unknown. Thus, a framework for quantitatively comparing such models is required and currently implemented within the framework of the International Soil Modeling Consortium. We propose to first use benchmark scenarios that test individual components of the complex models, such as root architecture, water flow in soil and roots, solute transport in soil and roots. While the latter will focus mainly on comparing numerical aspects, the root architectural models have to be compared at a conceptual level as they generally differ in process representation. Therefore, we suggest experimentally observed reference root systems against which model outputs can be compared. In a second step, benchmarking scenarios for the coupled root-rhizosphere-soil models are defined. This will help to quantify how the spatiotemporal development of the root architecture affects the spatiotemporal organization in the rhizosphere. All benchmark scenarios and model intercomparisons are presented in Jupyter Notebooks for transparency and future accessibility. Results of step 1 are expected to help interpret differences found in step 2. We expect that this benchmarking will result in improved models, with which we can simulate various scenarios with greater confidence, avoiding that future work is based on accidental results caused by bugs, numerical errors or conceptual misunderstandings and will set a standard for model development.

16:15 - 16:30

INFLUENCE OF ROOT MUCILAGE GEL PROPERTIES ON PORE-SCALE PROCESSES IN THE RHIZOSPHERE

M Brax¹, G Schaumann¹, D Diehl¹

¹*University Koblenz-Landau, Institute for Environmental Sciences, Group of Environmental and Soil Chemistry, Landau in der Pfalz, Germany*

The exudation of mucilage as a gel-like substance by plant roots appears to be a strategy for plants to overcome drought stress as it affects soil properties in the rhizosphere. Those strategies include an increase of soil water content and a decrease of the hydraulic conductivity toward the roots. The modulation of soil properties in the rhizosphere results from pore-scale processes probably also induced by mucilage. However, most of these processes remain unproven due to the lack of methods for *in situ* detection of mucilage and due to the lack of knowledge concerning the properties of interparticulate mucilage. Furthermore, it is not clear how differences in the chemical structure and composition of various root mucilages affect the gel properties and hence soil properties.

Our main objective was to elucidate mucilage's interparticulate gel properties in order to clear the pore-scale processes leading to changes in the rhizosphere properties induced by mucilage. For this, we investigated how several chemical conditions including Ca²⁺ content, uronic acids, their degree of esterification and high molecular weight substances control the gel formation mechanism and gel properties in wheat and maize root mucilage, chia seed mucilage and model substances. Further, water mobility and microstructural stability were measured in water-saturated mucilage-amended soil samples with ¹H NMR relaxometry and rheometry respectively and the structure of the interparticulate polymer network was characterized with the electron microscope.

We found that the gel concentration, the gel formation mechanism, the amount and type of high molecular weight substance as well as the hard boundary constraint in soil pores and soil particles size were decisive factors affecting the organization, rigidity and gluing of interparticulate gel polymer network. Those, in turn,

can explain how the hydraulic conductivity, the microstructural stability and the water mobility change according to mucilage and to soil particle size.

16:30 - 16:45

ROOT POSITION AND TRAIT SHIFTS DEPENDING ON A NEIGHBOR'S NUTRIENT-ACQUISITION STRATEGY IN KWONGAN NUTRIENT-IMPOVERISHED SOILS

P de Britto Costa^{1,2}, RS Oliveira^{1,2}, E Veneklaas², H Lambers²

¹*University of Campinas , Campinas, Brazil*

²*University of Western Australia, Perth, Australia*

In kwongan heathlands, we find high diversity in species and nutrient-acquisition strategies. A mechanism that enables species coexistence in these places is facilitation of phosphorus (P) acquisition, mainly involving species with contrasting nutrient-acquisition strategies. However, the mechanism that underlies facilitation is still unknown. We aimed to investigate the changes in root architecture when plants were growing with facilitating neighbors. We compared root traits, root positioning, leaf tissue nutrient concentrations and biomass allocation when *Hibbertia racemosa* (focal species, mycorrhizal, non-cluster rooted) grew with *Banksia attenuata* (non-mycorrhizal, cluster-rooted), and when it grew with conspecific individuals. Plants were grown in 60 cm deep, 40 cm wide rhizoboxes filled with sandy nutrient-impooverished soil, and roots were observed over time and measured at harvest. When grown with only conspecifics, *H. racemosa* segregated their roots from other individuals. In contrast, when grown with *B. attenuata*, *H. racemosa* aggregated their roots with *B. attenuata* roots. Additionally, the focal plants invested less in root biomass, showed higher root specific length and positioned a greater proportion of their roots in the 20 cm top soil when grown next to an individual of *B. attenuata* than when grown with a conspecific. Also, there was a correlation with focal plant root position and presence of cluster roots of *B. attenuata*. However, nutrient concentrations in the leaves were not higher when *H. racemosa* grew with *B. attenuata*. Our results demonstrate that, rather than a random root positioning in soil, focal plants directed their rootgrowth towards the facilitating neighbor's roots, modifying root traits and investment, and that the main cause was *B. attenuata*'s ability to mobilize nutrients in nutrient-impooverished soils. We conclude that root positioning is a key mechanism for facilitation between neighbors of contrasting nutrient-acquisition strategies.

Chair: Steve Siciliano

15:15 - 15:45

RHIZOBACTERIAL COMMUNITIES ASSOCIATED WITH FLOWERING DESERT PHENOMENON (ATACAMA DESERT, CHILE) AND THEIR POTENTIAL AS PLANT GROWTH-PROMOTING BACTERIA

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The flowering desert (FD) phenomenon consists of the rapid flowering of a wide variety of native plants in the Atacama Desert of Chile, often considered the driest desert in the world. While studied for some time due to its beauty, our knowledge of plant-microbe interactions that occur during FD remain unknown. In this study we investigated the rhizobacterial community composition of *Cistanthe* sp., a representative FD plant, by using high-throughput sequencing (HTS) of 16S rRNA genes. Sampling was done during two FD events occurring in 2014 and 2015. HTS revealed that *Proteobacteria* (35 to 56%) and *Actinobacteria* (34 to 35%) were the dominant taxa, in both years respectively. An additional analysis of the rhizobacterial community of *Cistanthe* sp. was also done during the pre- (PF) and full-flowering (FF) periods in 2017. Results also revealed that *Actinobacteria* (50 to 59%) and *Proteobacteria* (15 to 19%) were the dominant taxa in the PF and FF periods. Principal coordinate analysis showed a higher grouping during PF compared with FF, while 28% (724) and 23% (603) of operational taxonomic units (OTUs) were unique to PF and FF, respectively. In contrast, 2,585 OTUs were shared between the PF and FF. Based on plant growth-promoting traits (including phosphorus solubilization, 1-aminocyclopropane-1-carboxylate deaminase activity, and production of auxins and exopolysaccharides), culturable bacteria were selected from rhizospheres during FD period in 2017. The influence of assembled bacterial consortia on tomato plant under water shortage stress was investigated. Inoculation of tomato seeds and seedlings resulted in a decrease in germination time, greater recovery of viable seedlings, and more plant growth under water shortage stress. Our results suggest that rhizosphere of *Cistanthe* sp. recruit specific bacterial community, which may contribute to plant growth during the FD phenomenon.

Acknowledgements: FONDECYT 11600302 and 11160112; INACH RT_06_17; Conicyt Scholarship 21151002.

15:45 - 16:00

FLAVONOIDS ARE INVOLVED IN PHOSPHORUS DEFICIENCY-INDUCED CLUSTER-ROOT FORMATION IN WHITE LUPIN

Mr. CY Xiong¹, Dr. LY Cheng¹, Prof. JB Shen¹

¹China agricultural university, Beijing, China

Initiation of cluster roots, which are induced by phosphorus (P) deficiency, in white lupin (*Lupinus albus*) requires auxin signaling, but its mechanism of regulation is unknown. Here, we found high flavonoid contents

in the cluster roots, which could negatively regulate auxin signaling as an auxin transport inhibitor, and thus hypothesized that flavonoids are involved in P deficiency-induced cluster-root formation by interacting with auxin. In this work, we tested the spatial-distribution of flavonoids in cluster roots, and found the local accumulation of flavonoids in cluster-root epidermis and primordium at similar sites with the distribution of auxin. Quantifying specific flavonoids in different segments of cluster roots at different growth stages showed a significant accumulation of naringenin and kaempferol in root tip compared with other root segments (juvenile, mature and senescence). Applying exogenous flavonoids to solution culture strikingly modified the cluster-root formation: naringenin and quercetin significantly increased primary root length by 19.0% and 34.1%; genistein and kaempferol significantly increased lateral root length by 48.7% and 49.4%; four flavonoids all significantly reduced lateral root density by 46.6% to 81.3%; naringenin and quercetin strongly inhibited polar auxin transport in hairy roots through transforming roots by a *DR5::GUS* reporter. The results suggested that P-deficiency signaling regulate cluster-root initiation via inducing flavonoid accumulation (especially naringenin) in root tip, which could be necessary for local alteration of polar auxin transport and subsequent auxin accumulation in root meristem during the early stages of cluster-root formation. This may imply an important role of flavonoids in regulating auxin signaling and programming cluster-root formation.

16:00 - 16:15

ROOT SYSTEM ARCHITECTURE AND SALT DYNAMICS AT THE ROOT ZONE

U Nachshon¹, L Anqi¹

¹*ARO Volcani Center, Rishon-LeTzion, Israel*

Soil salinization is a global concern with a great deal of importance to agriculture, especially in arid and semi-arid areas. While the impacts of soil properties and irrigation on soil leaching and salt removal from the root zone are well understood, our understanding of water-root-soil interactions under saline conditions is more limited.

This work explored the impact of the root system architectural properties on solute transport and salt accumulation in the sensitive and important region of the root zone. Research hypothesis was that plants with wide and well-developed root systems have an advantage under conditions of irrigation with water containing salts since a wide root system improves efficiency of soil leaching, reduces salt accumulation and lowers soil bulk salinity. Exploration of these processes was done by using different introgression lines of tomatoes (a cross between M82 and Penelli) that have variations in the dimensions, depths and shapes of the root systems, while maintaining relatively similar water consumption and transpiration rates. The different plants were grown in Hele-Shaw cells that enable to observe the growth of the root system, together with measuring soil salinity and water content.

The results have supported the research hypothesis, as it was observed that plants with wide root systems are in a better condition compare to plants with small root systems, under conditions of salty water irrigation. Soil measurements at the root zone have shown that for a greater root system the sensed salinity by the roots is reduced and that a better leach of the salts downward is achieved. The research has shed new light on the nature of water-root-soil interactions under saline conditions and it is suggested that in the future, controlling of the root system architecture may be used as a tool to improve crop growth under saline conditions.

16:15 - 16:30

RHIZOSPHERE MICROBIOME OF HALOPHYTES AS A SOURCE OF OSMOREGULATORY GENES

KA Malik², S Mukhtar^{1,2}, S Mehnaz²

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²*Department of Biological Sciences, Forman Christian College (A Chartered University), Lahore, Pakistan*

Rhizosphere microbiome plays a significant role in the functioning of plants and provides information regarding plant physiology. Bacterial plasmids carry genes that codes for additional traits such as osmoregulation, CO₂ fixation, antibiotic and heavy metal resistance, root nodulation and nitrogen fixation. Plasmid conferring osmoregulatory genes from halophilic rhizobacteria can be used to develop salt tolerant transgenic crops. Main purpose of this research was to study plasmid-conferring osmoregulatory genes in rhizospheric bacteria isolated from *Salsola stocksii* and *Atriplex amnicola*. A total of 45 halophilic bacterial strains from the rhizosphere of *Salsola* and 38 isolates from *Atriplex* were identified by using 16S rRNA gene analysis. Phylogenetic analysis showed that bacterial isolates belonging to *Bacillus*, *Halobacillus* and *Kocuria* were dominant in the rhizosphere of halophytes. Plasmid curing of isolates was done by heat shock method to study the effect of plasmid conferring salt tolerance. These plasmids were isolated and transformed into *E. coli* and growth response of original strains and transformed *E. coli* was compared at 1.5-4M NaCl concentration. Almost all strains showed optimum growth at 1-3.5M NaCl. These strains were related to *Bacillus* spp., *Oceanobacillus* sp., and *Halomonas* spp. Results of plasmid curing showed that some halophilic bacterial strains such as *Bacillus* strain HL2HP6 and *Oceanobacillus* strain LK3HaP7 lost their ability to grow in halophilic medium but they grew well on LB medium. Some plasmid cured strains also showed a change in sensitivity to specific antibiotics. Plasmids from *Bacillus* strain HL2HP6 were sequenced by using high throughput sequencing approach. Functional analysis of plasmid sequences showed different proteins and enzymes involved in osmoregulation of microorganisms, such as carbohydrates trehalose, cellobiose and sucrose dehydrogenases, inorganic ion transporters, ectoine synthetase, Porins OmpC and OmpF and different amino acids, such as proline, alanine, threonine and cysteine dehydrogenases and peptidases.

16:30 - 16:45

BACTERIAL COMMUNITIES ASSOCIATED WITH THE NATIVE ANTARCTIC GRASS DESCHAMPSIA ANTARCTICA.

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¹*Institute of Biochemistry and Biophysics Polish Academy of Science, Warsaw, Poland*

²*Warsaw University of Life Sciences, Warsaw, Poland*

Deschampsia antarctica is one of two flowering plants native to Antarctica. As most plants on Earth were recognized to form symbiotic relations with soil-dwelling bacteria, the rhizosphere of this Antarctic grass has also been investigated in such regard. Three distinct habitats within the *D. antarctica* root-associated bacterial communities were analyzed: the rhizosphere (root-adjacent soil particles), the epirhizome (root-bound bacteria) and the endorhizome (inner-root tissue bacteria). Phylogenetic analysis of the bacterial assemblages residing in those habitats has revealed the presence of several phyla, most notably Bacteroidetes and Proteobacteria. A clear distinction was apparent when the physiological properties of pure bacterial strains

isolated from each of the zones were analyzed. Rock-phosphate solubilizing abilities were a domain of the rhizosphere bacteria (providing dissolved mineral compounds for plant growth) as well as phytohormone production abilities (increasing root range and biomass). Epirhizome bacteria had biofilming properties (for root surface attachment) and displayed antifungal potential (chitinase production) whereas endorhizome isolates secreted cellulolytic enzymes (presumably for root penetration), produced phytohormones and provided reduced nitrogen compounds via N₂-fixation. This work was supported by the National Science Centre Poland (NCN) grant number 2016/21/N/NZ9/01536.

16:45 - 18:00 POSTER SESSION

(Foyer & Salon A, D, E)

08:30 - 09:30: KEYNOTE ADDRESS - YAKOV KUZYAKOV (SALON B+C)

MICROBIAL HOTSPOTS & HOT MOMENTS IN THE RHIZOSPHERE

Y Kuzyakov¹

¹Göttingen, Göttingen, Deutschland

The rhizosphere is distinguished from root-free soil by very high microbial activities, converting it to a hotspot of various processes. These activities include microbial growth, a broad range of enzyme activities, oxygen consumption and CO₂ production, turnover of all organic substances, mobilization of nutrients, etc. The main reason for these high activities is that microorganisms experience localized alleviation of C and energy deficiency, due to the release of easily available organic substances by the root. In this talk, the previously developed general concepts of microbial hotspots and hot moments will be elaborated for the rhizosphere.

A broad range of visualization approaches allowed quantification of rhizosphere hotspot properties and the assessment of their lifetimes. Based on ¹⁴C imaging, root exudates are localized at root tips and occupy not more than 10% of the soil volume. Despite their short lifetime of about 1-2 weeks, exudation hotspots trigger a cascade of microbial processes: microbial growth, flush of O₂ consumption and CO₂ release by fast decomposition of organics, and enzyme production and subsequent mineralization of N and P from SOM. All these processes structure the environment around the roots and make it more suitable for plant growth. Consequently, roots function as ecosystem engineers, building their living environment through close interactions with rhizosphere microorganisms. Generalized results from various non-destructive visualization approaches showed the rhizosphere extending 0.5-4 mm from the root surface, depending on the property. Only very few properties (O₂ and CO₂ concentrations, pH and water gradients) have ranges larger than 4 mm. Based on changes of enzyme activities in the rhizosphere compared to the bulk soil, we concluded for the first time that the C and nutrient limitations are hotspot specific and will be modified during the hot moments. This is one of the basic principles of root-microbial interactions in the rhizosphere.

09:30 - 10:00 POSTER PITCHES (SALON B+C)

10:00 - 10:30 HEALTH BREAK & POSTERS & EXHIBITS (FOYER & SALON A, D, E)

10:30 - 12:30 PARALLEL SESSIONS

Session Chair: Louise Nelson

10:30 - 11:00

MICROBE-TO-PLANT SIGNALS: A WAY TO DEVELOP CLIMATE CHANGE RESISTANT AGRICULTURE

D Smith¹

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Climate change will result in more stressful plant/crop growing conditions more often. It is becoming clear that members of the holobiont regulate and coordinate each other's activities through production of signal compounds, sometimes effective at very low concentrations. These are, effectively, the hormones of the holobiont. There are already a few examples of plant-to-microbe signals, microbe-to-microbe signals and microbe-to-plant signals. We have been working extensively with two microbe-to-plant signals: lipochitooligosaccharides and thuricin 17. They are able to exert large effects on plant stress responses and promote plant growth as a result. Under levels of stress that normally cause large reductions in plant growth, application of these signals can restore near-full to full growth. They do this at very low concentrations (10^{-6} to 10^{-8} M for LCOs and 10^{-9} to 10^{-11} M for thuricin 17). We have been able to show that these compounds can reverse the effects of abiotic stresses such as low temperature, drought, high temperature and salinity. It seems very likely that many more such inter-organismal signals will be discovered within the context of the holobiont. These can then be characterized as potential enhancers of plant development, growth and overall productivity. Such compounds have considerable potential in an agricultural context. The microbe-to-signal compounds represent a way to manage stress effects on crop plants that is biological, low-input and low cost. These inputs will be able to assist with management of stresses such drought, high temperature and salinity, providing a sustainable way to develop more climate change resilient agricultural production systems.

11:00 - 11:15

DEVELOPING A TRAIT-BASED FRAMEWORK FOR PREDICTING MICROBIAL RESPONSE TO EXTREME CLIMATE EVENTS

O Nicolitch¹, C Weser¹, CG Knight¹, RI Griffiths², T Goodall³, FT de Vries⁴

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⁴*Institute for Biodiversity and Ecosystem Dynamics, Amsterdam, Netherlands*

Soil microbial communities are key actors of nutrient and carbon cycling and thus support plant health and productivity. Extreme climate events such as drought, which are becoming more frequent with climate change, can strongly impact soil microbial communities, with implications for their functioning. However, while the effects of drought on soil microbial communities and their functioning are well-studied, we have limited knowledge on the effects of other extreme events that will increase in frequency, such as flooding heat waves, and freezing. We addressed this knowledge gap by developing and testing a unified trait-based framework for

predicting microbial community response to extreme events and the consequences for soil functioning. We hypothesised that specific microbial traits ('response traits' - resistance) underlie the ability of microbes to cope with extreme events, while microbial 'effect traits' (resilience) predict the consequences of microbial community change for soil functioning. Soils from 30 grassland sites across a European climatic gradient were subjected to a two-week drought, flooding, heating, or freezing event under controlled conditions. The response of microbial communities and their functioning was assessed during a one-month recovery period through a combination of enzymatic assays, 16S and ITS amplicons sequencing and shotgun metagenomic sequencing, alongside soil respiration measurements and basic physicochemical properties. Preliminary findings revealed a contrasted effect of climate disturbance on soil ecosystem functions depending on the climate of origin. Moreover, alteration of carbon and nitrogen cycles was dependent on the type of disturbance, with a particularly strong effect of heating.

11:15 - 11:30

PSEUDOMONAS SP. STRAINS MRBP4 AND MRBP13 ISOLATED FROM MAIZE RHIZOSPHERE SOIL, EXTENUATES DROUGHT STRESS IN INOCULATED MAIZE (ZEA MAYS L.) PLANTS

OB Ojuederie¹, OO Babalola¹

¹North-West University, Mafikeng, North-West Province, South Africa

Global changes in climatic conditions due to anthropogenic activities, has negative implications on food security and sustainable agriculture. Drought a prominent abiotic stress, reduces the photosynthetic machinery of plants leading to the over production of reactive oxygen species. This damages cellular membranes, and inactivates macromolecules and in severe cases leads to plant death. The rhizosphere microbiome consists of a diversity of microbial consortium of bacteria, fungi viruses and archea interacting with the soil and plant roots. Understanding the architecture and function of the activities of the rhizosphere, will permit the utilization of plant-microbe interaction for the promotion of plant growth and development, while mitigating the effects of climate change. Harnessing the beneficial plant growth promoting rhizobacteria in the maize rhizosphere soil could aid improvement of maize production cultivated in arid and semi-arid regions. This study therefore investigated the drought stress mitigation abilities of two isolates of rhizobacteria MRBP4 and MRBP13, identified as *Pseudomonas* sp. based on 16Sr RNA gene sequencing when inoculated singly or in combination on maize plants. Both strains possessed plant growth promoting traits and had the ability to tolerate high temperature of 50°C and low water activity of 0.919A_w. Co-inoculation of the strains significantly (p<0.05) enhanced plant biomass and physiological functions of maize plants under drought stress. The use of rhizobacteria strains with multiple plant growth promoting traits as biofertilizers should be considered as a bio-based approach for enhancing maize production under water deficit conditions.

11:30 - 11:45

RESPONSE OF THE WHEAT MICROBIOME TO THREE YEARS OF RAINFALL MANIPULATIONS

H Azarbad¹, A Agoussar¹, J Tremblay², E Yergeau¹

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²*Energy, Mining and Environment, National Research Council Canada, Montréal (Québec), Canada*

Although water stress resistance is traditionally thought to be encoded in the plant genome, relationships with microbes can also enhance plant tolerance to water stress. However, it is not known how this microbial facilitation develops through time and if it is transmitted from one generation to another. We hypothesized that plants exposed to water stress will associate with different microorganisms than plant not exposed to stress, and that some of these organisms will be transmitted to the next generation through the seeds. To test this hypothesis, we designed a multi-year field experiment where drought-tolerant and sensitive wheat genotypes were grown in a Quebec field that was never subjected to water stress. Soil water content (SWC) was passively manipulated using rain-out shelters removing 25%, 50%, and 75% of the precipitation. Seeds were harvested at the end of the growing season and seeded in the exact same plots the following year. DNA was extracted from soils before seeding (T0) and root, leaf, rhizosphere and seeds during each growing season and subjected to amplicon sequencing (16S rRNA gene and ITS). We present here the results from the first three years of the experiment (2016, 2017 and 2018). Wheat microbiome responses to the decreasing soil moisture were influenced by plant genotype and varied significantly through growing season and years. Our results demonstrated a clear differentiation in seed-associated microbiomes based on the genotype's sensitivity to water stress and on SWC. These differences tended to increase over the years, suggesting some level of adaptation to lower soil moisture through the seed microbiota. Our results demonstrate that there is some level of transmission of the wheat microbiota through different generation, and that the microbiota transmitted is influenced by the plant genotype and the soil microbiota, but also by the environmental conditions to which the plant is subjected.

11:45 - 12:00

THE EFFECT OF STRIGOLACTONES ON THE MICROBIOME OF RICE

B Kim¹, A Zancarini¹, H Bouwmeester¹

¹*University of Amsterdam, Amsterdam, Netherlands*

Strigolactones are endogenous plant hormones regulating plant development and they are exuded by the roots when plants experience nutrient deficient conditions. The latter promotes the mutualistic association with arbuscular mycorrhizal fungi that help the plant to uptake valuable nutrients from the soil. This shows that plant actively establish symbiotic interactions with microbes to overcome inadequate nutrition and that strigolactones play an important role in this. Despite this important role as rhizosphere signaling molecules, the effect of strigolactones on the global root and rhizosphere microbiome remains poorly understood. Therefore, we conducted metabarcoding analysis on 16 rice genotypes quantitatively differing in strigolactone production to elucidate if bacteria and other fungi respond to strigolactones. Using multivariate analyses, we were able to pinpoint distinctive differences in the microbiome composition depending on strigolactones exudation. Using

multivariate regression modeling and machine learning analysis we could show that several groups of microorganisms correlate with strigolactones. This suggests that strigolactones also play a role in the recruitment of other players in the microbiome. Further studies will assess the functional role of these microbiome changes and will further unravel this new role for strigolactones as a rhizosphere signaling molecule.

12:00 - 12:15

THE ROLE OF ARBUSCULAR MYCORRHIZA IN ADAPTATION TO ABIOTIC STRESS IN WHEAT

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The increase in frequency of extreme climate events due to anthropogenic climate change means that crops will be subjected to more than one abiotic stress event during development, finally affecting crop production and food security. As wheat is an important crop worldwide, development of low-input crop management strategies e.g. by exploiting ecosystems services, are important to counteract decreased yield and quality. Arbuscular mycorrhiza; a mutual beneficial symbiosis between roots of the majority of plant species and arbuscular mycorrhizal fungi, has shown to be an ecosystem service that can increase many plant species tolerance to both abiotic and biotic stress.

The aim of recent studies was to elucidate the possible role of arbuscular mycorrhiza on wheat tolerance to various abiotic stress types, focusing on drought-, heat- and salt stress.

In this endeavor, a series of semi-field experiments were performed in order to study how arbuscular mycorrhiza influenced growth and physiology of wheat as measured by photosynthesis, stomatal conductance, transpiration, chlorophyll fluorescence, plant nutrient uptake and allocation, as well as growth and yield.

The results showed that arbuscular mycorrhiza could increase tolerance of wheat to the abiotic stress types; drought, heat and salt. However, the influence of arbuscular mycorrhiza on wheat tolerance to drought stress depended on wheat cultivar.

This work indicates that, in breeding of cultivars for sustainable wheat production under climate change conditions, it may be relevant to include the phytobiome, in particular arbuscular mycorrhiza, for increased tolerance against abiotic stress.

12:15 - 12:30

MICROBIAL COMMUNITY IMPACTS PLANT PERFORMANCE BASED ON DROUGHT HISTORY

J Munoz-Ucros¹, RC Wilhelm¹, DH Buckley¹, TL Bauerle¹

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The mechanistic effect of rhizosphere interactions on plant productivity is an especially important consideration in light of recent climate change, where droughts are predicted to increase in frequency, severity, and range. Our aim is to determine the extent to which plants can recruit beneficial microorganisms, the mechanism by which the recruitment happens, and the contribution of those microorganisms to plant performance, when the system is under drought stress. We grew willow cuttings from elite cultivars in the greenhouse either under

well-watered or drought-stressed conditions, characterized their root exudates with HPLC, and collected the rhizosphere as an inoculum for consecutive tests. Using a single willow cultivar, we grew cuttings in three microbial communities, each of which had a different history of water stress, and subjected half of the plants to intense drought and recovery cycles. We found that root exudate profiles are characteristic of each species in the controls, and changed significantly in response to drought stress. By keeping all else constant, we found that the microbial community has significant effects on plant performance (namely growth and wood density). Data from high-throughput sequencing of the rhizosphere will allow us to characterize the changes in microbial community composition. Ultimately, we aim to provide novel insights into how agricultural systems will respond to new water regimes in the face of a dramatically changing climate.

SESSION 8: CARBON DYNAMICS IN THE RHIZOSPHERE

(GALLERY B)

Session Chairs: Bobbi Helgason & Jemaneh Habtewold

10:30 - 10:45

HOT OR NOT? EFFECT OF RHIZOSPHERE HOTSPOTS ON TOTAL SOIL RESPIRATION

M Holz¹, JN Becker²

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Soil-C efflux is a major pathway in the global C cycle. It is tightly linked to the rhizosphere, where soil microorganisms rapidly decompose C released from roots as root exudates. Recent application of imaging approaches have greatly improved our understanding of small-scale heterogeneity of C turnover in the rhizosphere and coined the term 'rhizosphere hotspots' for highly active areas. However, despite often assumed, the effect of these hotspots on total soil C balances is still unknown.

To bridge the gap between small-scale rhizosphere processes and individual plant-soil interaction, we grew 17 maize (*Zea mays*) plants in rhizoboxes filled with sandy field soil. After four weeks, each box was placed into an airtight plastic bag and CO₂ efflux was quantified using NaOH traps. The plants were labelled with ¹⁴C and C allocation to roots and rhizosphere was visualized and quantified by ¹⁴C-imaging. Enzyme activity (β -glucosidase) was measured by soil zymography, while ¹⁴CO₂ and total CO₂ efflux were quantified over 8 days. Image analysis for hotspot quantification of root exudation and enzyme activity was done using Matlab.

Activity of ¹⁴C-hotspots was positively related to ¹⁴CO₂ efflux ($r_{\text{root}}=0.58$; $r_{\text{rhizo}}=0.51$) and total CO₂ production ($r_{\text{root}}=0.34$; $r_{\text{rhizo}}=0.17$). A negative relation was found between β -glucosidase-hotspots and total CO₂ and the metabolic quotient of microbial C. Our results indicate a reverse effect of root exudates and enzyme activity on CO₂ production. While ¹⁴C-hotspots exert a direct positive effect on soil respiration, the opposite is true for soil enzyme activity. Reduced plant derived C in the rhizosphere may fuel enzyme activity due to the microbial need for C, favoring microorganisms with high carbon use efficiency. We propose that ¹⁴C-hotspots can be used as a proxy for CO₂ production and that a decline in root exudates may decrease soil CO₂ efflux due to a shift in microbial community composition.

10:45 - 11:00

IMPACT OF ROOT HAIRS AND TEMPERATURE ON MICROBIAL CARBON UTILIZATION AND ENERGY PRODUCTION IN THE RHIZOSPHERE

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Root exudation is controlled by two main factors: 1) nutrient deficiency leading to root hair proliferation and increased exudation, and 2) warming that enhances membrane permeability and so, exudate release as well as microbial substrate utilization and enzyme activities. The interactions of both factors: root hair induced exudation and faster microbial turnover are especially important in the rhizosphere. To clarify these interactions, two maize varieties – wild type (with root hairs) and mutant type (without root hairs) – were grown at 20 and 30°C for 2 weeks. For the first time we combined of two imaging techniques, zymography and planar optode with calorimetry-respirometry to localize hotspots of enzyme activity (β -glucosidase) and relate it to the spatial distribution of CO₂ production. This approach enabled to illustrate role of root hairs on carbon utilization and energy production in rhizosphere in response to warming.

Rhizosphere size for both enzyme activity and respiration was 30-50% broader in presence of root hairs, while boundaries of CO₂ were two times larger than enzyme activity. CO₂ concentration around root tips was higher than mature parts in both varieties. The overlapping of zymograms with CO₂ images showed 60% positive correlation between their hotspots localization – which were mostly associated with roots.

The effect of root hairs on enzyme activity and energy flow was negligible at 20°C. At 30°C however, β -glucosidase activity of wild type was higher than that of the mutant. This increase was accompanied by a decrease in fraction of growing microbial biomass and activation energy (enzyme efficiency increased). Thus, low amounts of exudates released by hairless maize results in shifts of microbiome and functions. Consequently, an enzyme pool with higher efficiencies – instead of higher enzyme expression– enabling metabolic needs to be met at lower costs was produced at higher temperature.

11:00 - 11:15

CARBON AND NITROGEN CYCLING IN THE RHIZOSPHERES OF DIFFERENT GRASSLAND SPECIES

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The conversion of dryland to irrigated grassland for high-intensity farming in New Zealand has led to increased nitrogen losses from soils through leaching and nitrous oxide emissions. Limiting the amount of nitrate that is formed in soil through nitrification has been identified as a key target to attenuate these losses. Plants can inhibit this process in their rhizospheres through root exudates by, inter alia, stimulating microbial nitrogen immobilisation. However, the extent to which different grassland species can influence nitrogen cycling in soils,

and the mechanisms they employ, are not well understood. The aim of this research was to examine the nitrification inhibiting capacities and the underlying mechanisms for common grassland species in New Zealand. Plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), perennial ryegrass (*Lolium perenne*), wild radish (*Raphanus raphanistrum*), and cultivated radish (*Raphanus sativus*) were grown under controlled conditions in a grassland soil and different rates of nitrogen were applied (0 or 550 kg urea-nitrogen/ha). Bare soil and bare soil with a known nitrification inhibitor (dicyandiamide) were subjected to the same nitrogen treatments, as positive and negative controls, respectively. Potential nitrification rates, different soil carbon fractions, and bacterial gene copy abundances were determined in the rhizosphere and control soil samples. Cold water-extractable carbon showed a negative relationship with the potential nitrification rates. The high nitrogen application rate increased the abundance of ammonia oxidizing bacteria in all treatments. The effect was lowest in the dicyandiamide and wild radish treatments, the abundances were approximately 1.7 times higher in the other rhizosphere samples. The results highlight the importance of plant-specific effects on linkages between carbon and nitrogen cycles in the rhizosphere.

11:15 - 11:30

COORDINATION OF SOIL MICROBES WITH ROOT FUNCTIONAL TRAITS AND IMPLICATIONS FOR SOIL RESPIRATION IN RIPARIAN AGROECOSYSTEMS

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Complex interactions underpin the functional linkages between root systems and soil microbial processes that drive soil respiration. Tracking root-microbe patterns is particularly important in agricultural landscapes where net flux of carbon to the atmosphere is of large concern. In this study, we used a functional trait-based approach to quantify belowground plant strategies in relation to microbial community structure in rhizosphere and bulk soil across riparian buffers in an agricultural landscape in southern Ontario. We deployed a field-based, root exclusion experiment to measure soil (soil + roots) and heterotrophic (root-free soil) respiration in situ and used qPCR to estimate genes and transcripts of the total bacterial and fungal communities. We maximized the range of belowground processes within the same watershed by sampling sites of distinct vegetative communities and variable soil properties. Paired sampling for root functional traits and microbial communities were compared to components of soil respiration and analyzed over the growing season. The percent contributions of heterotrophic respiration to total carbon dioxide fluxes from soil ranged between ~40 and 95%. Multivariate analysis revealed a dominant coordinated root trait axis, as would be hypothesized for a root economics spectrum, with roots of higher specific root length, specific root area, root nitrogen content (acquisitive strategy) in opposition to roots with greater root diameter, root tissue density, and ratio of carbon to nitrogen (conservative strategy). Microbial abundance and activity in soil were unrelated to this trade off, while microbial communities in rhizosphere soil were more strongly coupled to root strategies. This study advances our understanding of root trait coordination with soil microbes, which is only now beginning to be understood, and

represents one of the first studies to examine interactions between root traits and trait syndromes with soil microbial communities to investigate variation in soil respiration.

11:30 - 11:45

MONITORING SPATIAL AND TEMPORAL CARBON DYNAMICS IN NODULATED ROOTS BY CO-REGISTRATION OF MAGNETIC RESONANCE IMAGING AND POSITRON EMISSION TOMOGRAPHY

R Metzner¹, D Pflugfelder¹, J Bühler¹, A Chlubek¹, S Jahnke¹, **R Koller¹**

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In terrestrial ecosystems plants have developed various mechanisms for symbiotic coexistence with root colonizing microorganisms in order to overcome plant nutrient shortage. In turn to this nutrient supply, plants allocate significant amount of photosynthetically fixed carbon (C) belowground, fuelling activity and growth of associated microorganisms.

Due to the opaque nature of soil, a direct observation of belowground processes is not possible and considerable uncertainty exists how plants orchestrate C allocation facing conflicting demands of C allocation towards beneficial microbial associations and building up plant structures. However, the application of non-destructive 3D-imaging methods including Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) offers new perspective in analysing belowground processes on individual plants. MRI allows for repetitive measurements of roots growing in soil and facilitates quantification of root system architecture traits while after labelling plant shoots with ¹¹C₂, the dynamics of long-distance C transport in plants can be analysed with PET.

We will present recent technological progress in repetitive MRI-PET co-registration enabling imaging of C tracer allocation at high spatial and temporal resolution along roots. We used *Pisum sativum* as a model for legumes and applied nitrate as an additional N source to the nodulated roots in order to quantify short term C allocation dynamics in the root system. We found that the fraction of ¹¹C tracer arriving in the most active nodules decreased by almost 40% and remained stable between 16h and 42h after the N application.

Our results highlights that the combination of MRI-PET enables deeper insights into short term C dynamics of roots and interactions with colonizing microbes. We expect that co-registration of MRI-PET has high potential for revealing mechanisms that relate to dynamic fitness traits and support breeding programs for future crops.

11:45 - 12:00

NANOSIMS: OPPORTUNITIES TO STUDY CARBON AND NITROGEN TRANSFORMATION AND CYCLING IN PLANT-MICROBIAL-SOIL SYSTEMS

XH He¹, GH Yu²

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²*Institute of Surface-Earth System Science, Tianjing, China*

Little is known about the spatial organization and functionality of soil microorganisms in the accumulation or transformation of soil carbon (C) and nitrogen (N) in an agricultural ecosystem. Recently, a novel imaging

facility, nano-scale secondary ion mass spectrometry (NanoSIMS), is capable of the concurrently quantitatively high spatial resolution imaging of five stable isotopes (e.g., $^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$, $^{34}\text{S}/^{32}\text{S}$, or any element in the Periodic Table) with high sensitivity at ≥ 50 nm metric and ppm atom scales. NanoSIMS analyses may be particularly powerful when combining with ^{13}C - and ^{15}N -labelled amino acids, which can bound to their amino, carboxyl or both groups with fluorescence colours depending on their size. The quantitative measurement of molecular and isotopic patterns of inorganic and/or organic C and N source could be hence imaged and detected. We applied NanoSIMS to examine submicron effects of inorganic versus organic fertilization on associations of organic and mineral components in a red soil in south China. Results showed that organic amendments in a 25-years long-term fertilization field have significantly increased the mineral availability, particularly in the short-range-ordered (SRO) phases. NanoSIMS images provided direct evidence that citric acid, a major component of root exudates, promoted the formation of SRO minerals, and that SRO minerals acted as "nuclei" for C retention, and that the submicron elemental distribution and spatial heterogeneity in the soil colloids and the ratios of $^{12}\text{C}-/^{27}\text{Al}^{16}\text{O}-$ and $^{12}\text{C}-/^{56}\text{Fe}^{16}\text{O}-$ were greater under organic fertilization than under chemical fertilization. The current progress and potential application of NanoSIMS is also generally discussed in recent studies on soil carbon and nitrogen cycling in plant-microbial-soil systems.

SESSION 9: RHIZOSPHERE PROCESSES FOR SUSTAINABLE AGRICULTURE

(GALLERY C)

Session Chair: Melissa Arcand

10:30 - 11:00

FUNCTIONAL RHIZOMICROBIOME: ROLE OF BENIGN BELOW-GROUND MICROBES IN MODULATING ABOVE-GROUND PLANT FITNESS AND SURVIVAL.

H Bais¹

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Plant microbiome research is at a point where there is great potential for significant advances. The positive impact of plant-associated microbes, especially in the rhizosphere, is well-established. Laboratory and field studies consistently document the growth, health and protective benefits conferred to plants by applying plant growth promoting rhizobacteria (PGPR). As the research progresses, we are gaining a greater depth of knowledge into the genetic and functional factors of plant host and rhizobacteria community interactions in the rhizosphere. Current focus is heavily reliant on defining and identifying a core microbiome, and common functional taxonomic units in beneficial plant-microbe associations. While this provides an invaluable foundation to our understanding of plant-PGPR interactions, in order to move the research to the next level, it will be important to describe and document the genetic and molecular mechanisms employed in these interactions. In this way, we will be able to restructure and harness these mechanisms in a way that allows for broad-based applications in agriculture.

11:00 - 11:15

DOES THE GENOTYPE OF WHEAT PLAY A ROLE IN SOIL STRUCTURAL DYNAMICS?

S Schmidt¹, S Ellis¹, S Mooney¹, J Atkinson¹, M Lark¹, M Martinez Araya¹, M Bennett¹, K Ritz¹

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For high crop productivity it is important to have a healthy and well-structured soil to ensure a sufficient water and nutrient supply to the plant. The architecture of a root system arises through interactions between the plant and the soil environment. Plant roots have can change their growth environment by restructuring the soil through mechanical action, exuding compounds that affect soil structure, and indirectly by modulating the activity of soil organisms. It is known that these effects differ markedly between different different plant species, but it is unclear whether genetic variation within species results in differing effects on soil structure. We explored this by determining the potential of a mapping line of wheat plants to alter their growth environment by changing soil structure. A total of 95 wheat lines were grown over a period of 3 weeks in a de-structured loamy clay sand. Soil aggregate size distribution, root and shoot dry-mass and rhizosheath mass were determined, and the soil microbial community phenotype via phospholipid fatty acid profiling. Changes in soil structure induced by the parent lines (Rialto and Savannah) after 2, 4, 6 and 15 days were investigated using X-ray Computed Tomography. Root, shoot and rhizosheath mass differed between the lines. Soil properties under Rialto were more similar to fallow soil than under Savannah. There was a larger proportion of aggregates <0.25µm in soil under Savannah than fallow soil and soil under Rialto. Soil under Savannah also showed a greater porosity and a higher pore connectivity compared to soil under Rialto or fallow. Rialto had greater root mass compared to Savannah but soil physical properties were more similar to soil under fallow. The bulk soil microbial phenotype did not differ between wheat lines. These early data suggest that the genotype of wheat can differ in its impact on soil structural properties.

11:15 - 11:30

BACTERIAL MICROBIOMES AND PLANT GENOTYPES: WHAT DRIVES PHENOTYPIC VARIATION AMONG CANOLA LINES?

SD Mamet¹, N Bazghaleh¹, J Bell¹, T Dowhy¹, Z Morales¹, Z Taye¹, S Williams¹, M Arcand¹, EG Lamb¹, M Links¹, I Parkin², SD Siciliano¹, S Vail², B Helgason¹

¹*University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

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Canola is one of the world's most important oilseed crops and the most profitable commodity for Canadian farmers. Improving crop yield is crucial for ensuring food security for future populations. Our goal is to help crop breeders identify microbial community characteristics that improve crop yield stability and agricultural sustainability. To achieve this, we conducted a field experiment in which 16 *Brassica napus* (canola) genotypes were planted in a 10-week replicated ($n = 3$) field experiment in central Saskatchewan at one location in 2016 and three locations in 2017. We sampled the DNA of root- and rhizosphere-associated bacteria weekly and assessed composition using high-throughput 16S rRNA gene sequencing. Our key hypotheses were: i) plant

development and environmental characteristics influence key bacteria in the canola microbiome; ii) this microbiome differs among canola genotypes; iii) these differences are driven by key bacteria (or bacterial guilds); and iv) yield variation may be explained more by microbiome and plant variation relative to environmental influences. Preliminary results suggest differences in the bacterial microbiome among lines were relatively consistent between years, particularly among root communities. Variance among genotypes appeared to be maximized during crucial phenological events, particularly flowering, and maturity. This presentation will expand on these results (i.e., hypotheses 1 and 2), as well as hypotheses 3 and 4.

11:30 - 11:45

ROOT-ASSOCIATED MICROBIOME OF MAIZE GENOTYPES WITH CONTRASTING PHOSPHORUS USE EFFICIENCY

EA GOMES¹, CA OLIVEIRA¹, UG LANA¹, SM de SOUSA¹, IE MARRIEL¹

¹*Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil*

Marginal soil fertility, soil acidity, aluminum toxicity, and low nutrient levels, especially phosphorus (P), are major limiting factors to maize production in highly weathered Oxisols that are prominent in the tropics. Plants have evolved several strategies to improve P acquisition, including the ability to associate with soil microorganisms that potentially enhance P uptake and plant nutrition. Previous results of our group showed that host genotypes and different types of fertilizers influence the composition of plant microbiomes. Using deeper next generation sequencing strategies we evaluated the influence of maize genotypes, phosphate fertilizers (soluble and rock phosphate) and P soil levels on the structure of maize rhizosphere and endophytic microbial communities. Our results suggested changes in microbial community composition associated with type and level of phosphate fertilization and host genotype. Whilst it is not possible to establish causality relations, our data highlights a few candidate taxa that could be involved in phosphate solubilization and plant growth promotion. This can represent a shorter path to further studies aiming the isolation and validation of taxa described possibly involved in rhizosphere soil P release and their use as bioinoculants. Hence, we established a collection of maize rhizosphere and endophytic microorganisms and part of this collection was characterized for the synthesis of phytohormones, siderophores, fosfatase, fitase, organic acids, drought tolerance, mineral solubilization of P and K, nitrogen fixation and biological control. The dominating genera were *Bacillus*, *Burkholderia* and *Azospirillum* and fungi genera *Aspergillus*, *Penicillium* and *Talaromyces*. Selected microorganisms with plant growth promoting characteristics were inoculated in maize plants cultivated in hydroponic conditions, greenhouse and field, resulting in increase of root and shoot dry weight, and N, P and K mobilization. This strategy may help in maintaining and improving grain productivity, environmental quality, reduced production costs and decreased use of chemicals fertilizers in agriculture.

11:45 - 12:00

THE RHIZOMICROBIOME OF SORGHUM: IMPACT ON PLANT GROWTH AND STRESS TOLERANCE

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¹*Netherlands Institute of Ecology (NIOO-KNAW), Microbial Ecology, Wageningen, Netherlands*

Sorghum is an economically important cereal crop used for animal feed and human food worldwide. Due to the high demand for its different uses, development of new sustainable strategies that improve or safeguard sorghum production is needed. These strategies not only encompass plant breeding and agricultural management practices, but also harnessing beneficial microbe-crop relations, which are key to the development of sustainable crop production. Despite the large number of studies addressing plant-microbiome interactions, little is known about the sorghum microbiome and how it affects sorghum growth and tolerance to biotic and abiotic stresses. The overall objectives of our research were to investigate the dynamics of the sorghum root microbiome and to explore the beneficial effects of the root microbiome on sorghum growth and stress tolerance. I will provide background information on the composition, spatial distribution and dynamics of the root microbiome and its importance for plant growth and health. Furthermore, I will present the role of root exudates in the recruitment of the rhizosphere microbiome and will discuss other drivers of rhizosphere microbial community assembly. Additionally, I will provide examples on how the root microbiome can provide tolerance to the host plant against abiotic disturbances, in particular drought. Finally, I will provide insights into how genetically microbial inoculants can impact plant growth and nutrient acquisition.

12:00 - 12:15

TWO DISTINCT GENE-COEXPRESSION NETWORKS, MYCORRHIZAL MODULE AND PHOSPHATE-STARVATION MODULE, ARE INDEPENDENTLY REGULATED IN THE FIELD

Y Sugimura¹, A Kawahara², H Maruyama¹, T Ezawa¹

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Inorganic phosphate is a macro nutrient that often limits plant growth due to its poor mobility in soil. Plants have developed diverse strategies for phosphate acquisition during evolution, e.g., lateral root formation, secretion of acid phosphatase and organic acid, and mycorrhizal formation. Although the regulatory mechanisms of these individual processes have been studied in lab experiments, we have little information about how plants orchestrate these processes with limited resources in the field. To this end, the field transcriptomics approach with gene-network analysis was employed. Maize roots and rhizosphere soils were collected across the US corn belt (66 plants) and across Japan (194 plants). mRNA was purified from the roots, sequenced on the illumina platform, mapped on the maize genome, and subjected to gene-coexpression network analysis. Sixteen coexpression modules were identified, e.g., those involved in water uptake, cell wall biogenesis, nitrogen assimilation, mycorrhizal formation/functioning, and phosphate starvation. In the mycorrhizal module (1,541 genes) the genes of mycorrhiza-specific transporters and enzymes for nutrient exchange were enriched, while in the phosphate-starvation module (214 genes) those encoding a high-affinity phosphate transporter, secreted acid phosphatase, and the proteins involved in the inositol hexaphosphate-

signaling pathway were enriched. The mycorrhizal module was upregulated with increasing mycorrhizal colonization and soil nitrate-phosphate ratios and downregulated with increasing leaf-phosphorus content, and the phosphate-starvation module also responded similarly to some of these factors. The two modules, however, were regulated independently, that is, the expression levels of the genes in the mycorrhizal module were not correlated with those of the genes in the phosphate-starvation modules. In the field, population density of mycorrhizal fungi and phosphate availability vary considerably due to, e.g., different soil chemical/physical properties and fertilizer management. It is likely that plants optimize resource allocation to these two modules to maximize the efficiency of phosphate acquisition in a given environment.

10:30-11:20 TECHNICAL SESSION: PreSens Precision Sensing, Germany (GALLERY D)
ILLUMINATE THE ROOT-SOIL INTERFACE. NON-INVASIVE IMAGING OF O₂, PH AND CO₂ IN SOIL SCIENCE.

12:30 - 14:00 LUNCH (CENTENNIAL HALL)

14:00 - 15:00 KEYNOTE ADDRESS - ETIENNE YERGEAU (SALON B+C)
UNDERSTANDING AND ENGINEERING THE RHIZOSPHERE MICROBIOME

E Yergeau¹

¹*Université du Québec, Institut national de la recherche scientifique, Institut Armand-Frappier, Laval, QC, Canada*

Plants and their microbiota form an inseparable entity known as a holobiont. The concept of the hologenome (all the genomes of the holobiont) as an evolutionary unit suggests three mechanisms that could be harnessed to rapidly evolve/adapt holobionts: 1) amplification/reduction of existing microbial partners, 2) recruitment of new partners from the environment, 3) recruitment of new genes (through HGT). For this presentation, I will discuss recent work from my group that aimed at understanding and manipulating the microbial part of the wheat holobiont for increased resistance to water stress. Soil water stress history was shown to be the dominant constraint on the response of the wheat microbiota to subsequent short-term water stress. With these results in hand, two experiments were carried out aiming at contrasting the “amplification/reduction”, “recruitment” and “HGT” mechanisms of rapid holobiont adaptation. The soils with the different histories were used as microbial or DNA inocula for wheat plants under water stress. The response of the wheat holobiont to water stress was mainly through amplification/reduction of existing microorganisms and genes, with a small part of the inoculated microorganisms and genes being stably recruited in the rhizosphere. Globally my research indicates that it is possible to modify the plant holobiont through microbiota manipulation, but that efforts should be focused on manipulating the initial communities as plant under stress will heavily rely on the already present microbiota. More research will be however necessary to completely understand the mechanisms involved and apply them for e.g. increasing crop yields, increasing resistance to stress or reducing human pathogens loads.

SESSION 10: CLIMATE CHANGE, ABIOTIC STRESS IN THE RHIZOSPHERE**(GALLERY A)***Session Chairs: Louise Nelson & Don Smith***15:15 - 15:30****DISENTANGLING THE ROLE OF ROOT TRAITS IN THE RHIZOSPHERE COMMUNICATION NETWORK AND THEIR RESPONSE TO CLIMATE CHANGE.****A Williams¹**¹*University of Manchester, Manchester, United Kingdom*

Understanding the chemical communication network between the plant and its root associated microbes presents a challenge due to the complexity and variability of the plant-soil interface. Using powerful mass spectrometry techniques (liquid, and gas, chromatography -MS) it is possible to produce a global, albeit variable, profile of the rhizosphere chemical structure. However, this approach does not directly permit the identification of exuded plant compounds, that is possible in hydroponics.

To address this, we have undertaken a concerted exploration of different root exudate collection techniques across a range of phylogenetically diverse grassland species, grown in natural soil. By cleaning and transferring plants to a hydroponic solution for recovery we were able to collect exudates and compare these to leachate. These data revealed distinct metabolic profiles of exuded plant metabolites and their biological fate in the rhizosphere, across the functional groups; forb, grass and legume. We tested the hypothesis that phenotypic markers of exudation quality and quantity can be predicted by structural and morphological root traits such as specific root length (SRL), root tissue density (RTD) and root tip number. In particular, we expected root traits associated with exploitative growth strategies, such as high SRL, to be linked to high rates of root exudation and shift in metabolic profile (potentially towards an increase in semio-chemicals such as flavonoids and organic acids).

The developed techniques can be subsequently employed to survey the impacts of environmental stresses (such as drought and elevated CO₂) on particular species, or species assemblages, and soil functioning. A novel approach synchronising MS data with rhizosphere microbiology community analysis will be presented that expands understanding regarding the complex chemical communication network that lead to specific microbial communities and soil function, in response to future climate change.

15:30 - 15:45**RESPONSES OF TREE INTERACTIONS TO CLIMATE CHANGE AND INVASIVE SPECIES****J Simon¹**¹*University of Konstanz, Konstanz, Germany*

Global change, such as climate change and/or biological invasions of species, affects plant fitness and competitiveness, especially in highly vulnerable seedlings, resulting in severe effects on forest ecosystems in

the future. Facing these challenges and the accompanying ecosystem changes, more detailed knowledge of the basic processes and underlying mechanisms is required to understand ecosystem functioning, and thus develop and evaluate strategies for sustainable ecosystem management to secure ecosystem services for modern society. Most studies on plant interactions with regard to nitrogen acquisition and/or partitioning have focused on inorganic N, and only recently organic N acquisition of plants has come into the focus of research. Furthermore, plants have developed different mechanisms to optimise the utilisation of limited nitrogen (N) resources; however, the complex interactions between different species, particularly long-living woody species, with regard to the competition for N in the rhizosphere are currently only little understood. The research presented here provides new insights into the understanding of the processes involved in the regulation of belowground competition for nitrogen in temperate forests. We studied different tree species and their N acquisition strategies (i. e. inorganic and organic N forms) in intra- and interspecific competition. Further results from experiments including important abiotic factors provide evidence that these N uptake strategies strongly depend on the environment. Moreover, N acquisition in the rhizosphere of forests can also be influenced by invasive tree species.

15:45 - 16:00

FINE ROOT CARBON EXUDATION AND MORPHOLOGY EFFECTS IN DIFFERENT NITROGEN SOURCES AND AIR HUMIDITY TREATMENTS

M Sell¹, P Kupper¹, G Rohula-Okunev¹, A Rezapour¹, K Parts¹, I Ostonen¹

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Rising atmospheric temperature, precipitation and therefore the increase of air humidity will have a significant influence in plants' growth and functioning in the near future. In the unique Free Air Humidity Manipulation experiment in Estonia, silver birch (*Betula pendula* Roth.) trees have shown to have a clear effect growing in elevated air humidity.

We aimed to compare root morphology response in controlled conditions to confirm the major effect of air humidity increase in the experimental site and to measure accompanying changes in fine root carbon exudation. To estimate the changes in fine and absorptive root morphology and carbon exudation rate, 2-year-old silver birch saplings were grown in PERCIVAL growth chamber with stable temperature, but different treatments such as air humidity (low, moderate, elevated) and in addition a treatment with different nitrogen sources – nitrate and ammonium. The collection of total fine root carbon exudation was conducted by culture-based cuvette method and total organic carbon content was determined by Vario TOC analyser (Elementar GmbH, Germany). In the field experiment the trees were forming longer and thinner short roots in humid conditions, in controlled experiment root morphology had similar response. Root carbon exudation was higher in elevated air humidity conditions, but the effect of different nitrogen treatments was negligible. The mechanisms of fine root morphological adaptations and associated change in carbon exudation in more humid conditions that may lead to essential changes in carbon allocation will be discussed. This study was supported by the Estonian Ministry of Education and Research (Personal research funding PUT1350).

16:00 - 16:15

RHIZOSPHERE LEGACY: AMELIORATION OF MICRO-BIOPHYSICAL PROPERTIES OF COMPACTED SOIL

BS Razavi¹, N Rudolph-Mohr², CC Tebbe³

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Soil compaction is a multi-disciplinary problem in which soil, plant, and air operations play an important role and may have dramatic environmental consequences throughout the world. In compacted soils, the increase in bulk density, and the accompanying decrease in porosity hinders the exchange of oxygen, carbon dioxide and other gases, thereby causing hypoxic stress in plant roots. Hypoxic stress can effects root physiological functions, reduce soil enzyme activity, hence reducing soil fertility. For the first time we applied a unique combination of two imaging techniques, zymography and optodes sensors with molecular microbial community analysis to illuminate the rhizosphere self-regulation for amelioration of biophysical properties of compacted soil. To this end maize in compacted and uncompacted soil under control condition for 2 weeks was planted. Soil oxygen map and β -glucosidase activity in compacted maize treatment overlaid with the extracted root system demonstrated more than 65% positive correlation between hotspots of enzymatic activity and localities with high oxygen concentration –which were mostly in association with root. Similarly, extend of rhizosphere for oxygen concentration and enzyme activity across the root of compacted soil was 1mm broader than the uncompacted.

Based on root morphology analysis, compacted maize reduced roots diameter and increased the distribution. Which resulted in 30% higher ratio of rhizosheath mass in compacted than uncompacted soil. Rhizosheath formation changed porosity and aggregation around the root, thus, improved oxygen exchange. Accordingly, bacterial abundance and alpha diversity in hotspots of compacted soils were higher than the one of uncompacted. Thus, microorganisms localized in hotspots (rhizosheath) respond to better aeration, new carbon inputs compared to those inhabiting in the bulk soil. This confirms the distinguished role of rhizosphere-self organization for enzymatic mobilization of nutrients, and point out on the importance of aeration for rhizospheric microbial functionality (such as, enzyme expression for nutrients mining).

16:15 - 16:30

PLASTICITY AS THE KEY ABILITY OF ROOTS FOR ADAPTATION TO VARIOUS TYPES OF WATER STRESSES IN RICE PLANTS

A Yamauchi¹, M Nakata-Kano¹, E Kameoka², R Suralta³, J Niones³, Y Inukai⁴, S Mitsuya¹, T Tran⁵, S Owusu-Nketia¹, D Nguyen⁵, Y Watanabe¹, T Kabuki¹, D Menge⁴

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Roots play important roles in crop adaptation to various abiotic stresses such as water stress. We have accumulated experimental evidences showing that a root system of an individual plant is an integration of component roots with dissimilar morphology, anatomy, and physiological functions including hydraulic conductivity. They are also different in developmental responses to various environments. The response of such root system to a certain soil environmental condition largely determines the plant's ability to adapt to the condition. The ability of plant to change its morphology, as environmental conditions change is defined as phenotypic plasticity. A series of experiments are now in progress to determine the functional roles of root plasticity by using various accessions/populations mainly in rice such as the *Oryza* SNP panel, chromosome segment substitution lines derived from Nipponbare and Kasalath cross, and Sasanishiki and Habataki, chromosome segment substitution lines with the genetic background of KDML105, IR 64 near isogenic lines, and a few promising genotypes including New Rice for Africa that is an interspecific cross between *Oryza sativa* and *Oryza glaberrima*. Root plasticity has been phenotyped by using a range of methods, including rootbox-pinboard method, slant tube method, line source sprinkler system which can create a gradient in drought stress intensities, and under field conditions using both monolith and core sampling. These results consistently showed that the root plasticity specifically in branching, deep rooting and the associated aerenchyma formation, which are exhibited in response to water stress, substantially contribute to dry matter production through enhanced water uptake. Genotyping is also in progress by using some of the population to locate genes responsible for the root plasticity traits. Further study is in progress to more precisely identify the quantitative trait loci responsible for the root plasticity and to examine the physiological function of such plasticity for plant adaptation and productivity.

16:30 - 16:45

ROLE OF ROOT PLASTICITY ON RESPONSE TO SOIL COMPACTION

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Compacted soils limit crop production and affect millions of hectares of agricultural land globally. Plants vary in tolerance to soil compaction and roots express various plasticity responses. Unfortunately, the relative importance of those responses for crop yield is practically unknown. We carried out a set of experiments and simulations to study the diversity and implications of root plasticity in a collection of sorghum genotypes. Plants were grown in a greenhouse for three to six weeks in homo- and heterogeneous soil density gradients (from 1.3 to 1.8 g/cm³). We used a combination of destructive and non-invasive phenotyping technologies, including automated shoot imaging and magnetic resonance imaging of roots. Leaf area, plant biomass and root length decreased under soil compaction, but significant genetic variation in plant plasticity to soil compaction was observed. Aboveground plasticity was correlated to plant size, with larger genotypes responding earlier and more strongly. Roots were more affected than shoots and plasticity was expressed mainly in fine roots (diameter < 0.2 mm). Impeded plants produced 10-25% less fine roots independent of aboveground plasticity responses. In heterogeneous compaction gradients, plants had more fine roots in loose compared to compacted layers (10-25% more); more than in homogenous loose soil treatments. We propose that these

responses are a resource optimization strategy, which plants use to proliferate more easily to penetrate soil layers. We conclude that the root responses to soil compaction are complex, involving both plasticity and size-correlated changes. We suggest that high proportions of thicker roots and compensatory growth are plastic adaptations to cope with compaction. This is relevant for future cropping techniques or breeding programs focused on minimizing yield penalties when root plasticity is exploited.

SESSION 11: NEGATIVE SOIL-PLANT-MICROBE INTERACTIONS

(GALLERY B)

Session Chair: Lori Phillips

15:15 - 15:30

UNRAVELING THE MECHANISM BEHIND PLANT AND BENEFICIAL MICROBES INTERACTION LEADING TO DISEASE SUPPRESSION

KH Hooshmand¹, EN Kudjordjie¹, RU Sapkota¹, MO Nicolaisen¹, IN Fomsgaard¹

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Plants at different growth stages continuously synthesize bio-active chemical compounds which serve as a direct protection against subsequent infection by detrimental pathogens. In addition, the bio-active compounds may be exuded to the rhizosphere where they facilitate the interaction between the plant and beneficial microbes, which then could contribute to suppression of soil-borne pathogenic microbes. However, the molecular mechanism underlying plant-microbe interaction associated with plant resistance against root diseases has been poorly elucidated. The aim of the present study was to investigate the functional role of root exudate (from different host genotypes) in mediating belowground indirect plant defense.

Arabidopsis thaliana wild type accessions (Columbia-0 and Landsberg erecta-0) were grown for 3 weeks in non-sterile soil. Subsequently, the root of each individual plant was challenged with *Fusarium oxysporum* matthioli pathogen. The root and shoot samples were collected at intervals of 5 days after infection for 25 days, lyophilized, extracted and were subjected to both targeted and untargeted metabolomics analyses (liquid chromatography tandem-mass spectrometry and gas chromatography–time-of-flight mass spectrometry platforms) as well as metabarcoding (Illumina MiSeq, for bacteria and fungi community profiling). For targeted analysis, the LC-MS/MS method for identification and quantification of intact glucosinolates, their degradation products, plant hormones, and mycotoxins was developed and fully validated.

Phenotypic characterization of the plants displayed pronounced disease symptoms (chlorosis development) for Landsberg erecta lines at different growth stages whereas Columbia-0 ecotype exhibited strong resistance to pathogen exposure. The disease progression was also evaluated on a molecular level and resulted in the identification of beauvericin in both root and shoot tissues of the susceptible plants which likely was associated with promoting pathogenicity in *Fusarium oxysporum*. The result from this study will highlight the role of novel natural products in shaping the distinct member of the microbial community leading to diseases suppression.

15:30 - 15:45

POSSIBLE ROLE OF BACTERIAL COMMUNITIES IN MODULATING ABUNDANCE OF COMMON SCAB PATHOGEN IN RHIZOSPHERE AND GEOCAULOSPHERE OF POTATO

C. Goyer¹, K. Nahar¹, B. Zebarth¹, D. Burton², S. Whitney¹

¹*Agriculture and Agri-Food Canada, Fredericton, New Brunswick, Canada*

²*Dalhousie University, Truro, Nova Scotia, Canada*

Pathogenic *Streptomyces* spp. cause common scab of potato resulting in significant economic losses. It was previously reported that the genetic background of potato cultivars influenced the abundance of pathogenic *Streptomyces* spp., with 5 to 6 times more abundant *Streptomyces* spp. in the rhizosphere soils of susceptible cultivars compared with tolerant cultivars. It is possible that the diversity of soil bacterial communities might influence the abundance of pathogenic *Streptomyces* spp., however this has not yet been investigated. This study evaluated the effects of potato cultivars on diversity of bacterial communities in three spatial locations, bulk soil (BS), rhizosphere (RS) and geocaulosphere (GS), over the growing season. The experiment was conducted in a field naturally infested with common scab located in Fredericton, NB, Canada. Two tolerant (Goldrush, Hindenburg) and two susceptible cultivars (Green Mountain, Agria) were planted in a randomized completed block design with four or six replicates. Soil samples were collected from BS, RS and GS soils in July and August of 2013 and 2014. The tolerant cultivar Hindenburg had a significantly greater Shannon index (H') compared to Agria or to both susceptible cultivars in 2013 and 2014, respectively. The β -diversity of the bacterial community was significantly different between years, dates within each year and among locations (BS, RS, GS) but not among cultivars according to a permutational multivariate analysis of variance. Soil pH, temperature, NH_4^+N , and clay content were the most significant factors explaining the variability in the bacterial community diversity in RS with 19.1 % of the variation explained by the first two axes of a distance-based redundancy analysis. These results suggested that differences in growth of pathogenic *Streptomyces* spp. among cultivars may reflect cultivar-specific effects on soil chemical properties rather than effects on soil bacterial communities.

15:45 - 16:00

ROOT ROT ALTERS THE STRUCTURE OF THE PEA ROOT AND RHIZOSPHERE MICROBIOME

Z Hossain¹, M Hubbard¹, Y Gan¹, LD Bainard¹

¹*Agriculture and Agri-Food Canada, Swift Current, SK, Canada*

Understanding how root and rhizosphere microbiomes are affected by plant health is vital for developing sustainable crop production systems. Here we studied the microbiomes of healthy and diseased pea (*Pisum sativum* L.) root and rhizosphere using amplicon metagenomic sequencing. Nine fields in Saskatchewan, Canada were sampled. At early flowering, four diseased and four healthy samples were dug up from each field. *Aphanomyces eutiches* was detected in both healthy and diseased samples. Bacterial and oomycete alpha-diversity (i.e., richness, Shannon index) was higher in diseased root and rhizosphere samples than their healthy counterparts, while fungal diversity was unaffected. This runs counter to the general expectation that healthy samples had higher levels of microbial diversity. The community structure of the root and

rhizospheremicrobiomes were also significantly affected by the health status of the plants, with bacterial communities exhibiting the strongest differences between healthy and diseased samples. Healthy samples had a higher relative abundance of Proteobacteria, Cantharellales, Mortierellales and Olpidiales, and lower abundance of Acidobacteria, Actinobacteria, Bacteroidetes, Verrucomicrobia, Hypocreales, Pythiales and Peronosporales. Overall, the microbiome structure of diseased samples were more predictable than healthy samples. They had a higher number of indicator species (42 diseased vs. 11 healthy) and more taxa exclusively detected in the core microbiome. Overall, our results show key differences between the microbiomes of healthy and diseased pea. Further research into determining whether differences in the microbiome influence pea susceptibility to root rot, as opposed to root rot inducing changes in the microbiome are merited. Combined with the current results, such information could help assess root rot risks and/or develop management strategies to improve disease suppressiveness of agricultural soils.

16:00 - 16:15

DEVELOPING BENEFICIAL RHIZOSPHERE INTERACTIONS TO SUPPRESS ABOVE GROUND INSECT PESTS

A Gaudin¹, C Casteel¹, R Vannette¹, J Schmidt¹, R Blundell¹, A Igwe¹, A Cheung¹

¹*University of California Davis, Davis, USA*

Soils are a major source of plant associated microbes and soil management strategies, through their impacts on soil living ecosystem, can have a large impact on the outcome of plant-microbe interactions. There is increasing evidence that rhizosphere microbes can influence above ground plant-insect interactions through changes in plant signaling and defense but the management approaches that promote beneficial microbial populations remain poorly understood. We conducted both on farm and lab studies to investigate how soil management and rhizosphere microbes impact tomato nutritional and defensive cues and the downstream impacts on beet leafhoppers populations dynamics and preference. Soil microbiome sequencing coupled with transgenic/mutagenic approaches and multi-model inference show that organic management promoted beneficial plant-microbe interactions which regulate host plant resistance mechanisms and attractiveness to insect pests. Systemic plant resistance were dependent on salicylic acid accumulation and rhizosphere microbial communities, including mycorrhizal fungi. Our results suggest that organically managed soils and the associated microbial communities play an unappreciated role in depressing plant attractiveness to pests and can potentially decrease the incidence of the BCTV transmitted by the leafhoppers throughout the tomato-dominated agricultural landscape. Fostering these beneficial interactions will help promote the delivery of key soil ecosystem services critical for sustainability and resilience of farming systems.

16:15 - 16:30

SOIL COMMUNITY SHAPED BY LONG-TERM ORGANIC AMENDMENTS COULD ALLEVIATE ABOVEGROUND HERBIVORY DAMAGE VIA ENHANCING RICE PLANT TOLERANCE AND RESISTANCE

M Liu¹, L Jiang¹, L Luo¹, Y Zhang¹, X Chen¹, Z Xiao¹, F Hu¹

¹*Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu Province, China*

The effects of soil amendments on the soil community and related nutrient cycling functions are increasingly recognized. However, there is a knowledge gap of the functional roles of soil community shaped by different soil amendments on plant performance. Our study aimed to explore whether and how soil biotic factors modulated by long-term organic or chemical amendments, in comparison to soil abiotic factors, would promote rice plant growth and its susceptibility to herbivorous pests. A reciprocal transplant experiment was designed to test whether the amendment-modified soil community would affect plant growth and further extend the belowground effects to spatially distinct aboveground pest performance. Soil community inocula obtained from organic- or chemical-amended soils were introduced to the sterilized soils of their own or counterpart. The experimental pots were transplanted with rice seedlings and were harvested three weeks after infesting herbivores (*Nilaparvata lugens*). We found that soil community increased plant tolerance and resistance with the magnitude of positive effects being higher for those community modulated by organic than by chemical amendments. The increased tolerance was associated with the increase in plant biomass, and the increased resistance was due to the decrease in plant nutrition (amino acids and sugar concentrations) and concomitant increase in secondary metabolites (phenolics and salicylic acid concentrations). Moreover, linear correlation analysis showed that rice grown in organic-amended soils tended to invest more energy to growth than secondary metabolism in the trade-off between rice tolerance and resistance, as a result of high resource availability in organic amendments. Our findings highlight the importance of considering synergistic effects of soil community with abiotic factors on plant growth performance for better understanding the mechanisms of ecological intensification impact as well as the potentials of managing targeted soil community to reduce chemical fertilizer and pesticide usage without sacrificing the crop productivity.

16:30 - 16:45

THE POTATO COMMON SCAB-INDUCING PATHOGEN STREPTOMYCES SCABIES DEGRADES SUBERIN, A RECALCITRANT PLANT BIOPOLYMER

C. Beaulieu¹, R. Jabloun¹, M. Khalil¹, A. Sidibe¹, S. Lerat¹

¹*Université de Sherbrooke, Sherbrooke/QC, Canada*

The potato tuber is covered by a periderm impregnated with suberin, a recalcitrant polymer composed of a polyaromatic domain covalently linked to a polyaliphatic moiety. The capacity of *Streptomyces scabies*, a common scab-inducing pathogen, to degrade suberin was investigated. *S. scabies* was incubated in the presence of suberin-enriched periderm and the material was periodically observed under electron microscopy. There was no evidence of morphological modifications in the first weeks of incubation but after 60 days, suberin lamellae became detached from the secondary wall and broken lamellae were released. The genome of *S.*

scabies encodes a potential suberinase, the protein Sub1, which has been shown to be specifically induced in the presence of suberin. The *sub1* gene was expressed in *Escherichia coli* and the recombinant protein Sub1 was purified. The enzyme was shown able to release fatty acids from this substrate. *S. scabies* did not only exhibit the ability to degrade the aliphatic part of suberin, the bacterium also catabolized the main cinnamic acids (*trans*-ferulic or *p*-coumaric acids) composing the polyaromatic part of suberin. A proteomics study revealed that degradation of these aromatic compounds occurs via the β -keto adipate pathway. *S. scabies* efficiently competed with other soil bacterial species when grown in the presence of suberin. Soil samples from a potato field were inoculated in a culture medium containing suberin as the sole carbon source and a metaproteomics approach was used to identify bacteria that develop in the presence of suberin. The normalized spectral counts of extracellular proteins produced by the bacterial community drastically decreased from day 5 to day 20 and then slowly increased, revealing a succession of bacteria among which *S. scabies* was found. While the biological degradation process of suberin is attributed to soil fungi, the involvement in this process of soil bacteria deserves further investigation.

SESSION 12: AMENDMENTS AND COMMERCIAL INOCULANTS

(GALLERY C)

Session Chair: Anton Hartmann

15:15 - 15:30

SALINITY STRESS TOLERANCE OF 'MEYER' LEMON TREES IMPROVED BY A CONSORTIUM OF FOUR ARBUSCULAR MYCORRHIZAL FUNGI (RHIZOPHAGUS INTRARADICES, FUNNELIFORMIS MOSSEAE, CLAROIDEOGLOMUS ETUNICATUM, GLOMUS AGGREGATUM)

S. Garcia¹, K. Rola¹, S. Inman¹, J. Pienaar¹, J. Racsko¹

¹*Mycorrhizal Applications, LLC., Grants Pass, OR, United States*

Application of mycorrhizal inocula in agricultural systems has become increasingly popular to improve crop performance. The efficacy of the inoculum in agro-ecosystems has to be assessed for the continued development of the inoculum production technologies. The goal of this study was to investigate the efficacy of MycoApply® inoculum in container-grown 'Meyer' lemon trees. Commercially available MycoApply® arbuscular mycorrhizal inoculum containing four arbuscular mycorrhizal species (*Rhizophagus intraradices*, *Funneliformis mosseae*, *Claroideoglobus etunicatum*, *Glomus aggregatum*) (trade name MycoApply Endo) were applied to the lemon trees at a rate of 6,000 propagules per tree. Single trees were planted into tote bags filled with 250 kg granular clay medium and grown under severe salinity stress (100 mM NaCl drenched weekly). ANOVA analysis was used to determine statistical significance at a P level < 0.05. MycoApply Endo inoculum was effective in mitigating the negative effects of salinity stress and increasing mycorrhizal colonization of the roots, vegetative growth, relative chlorophyll content, yield, fruit size, photosynthesis, and chlorophyll fluorescence. Differences in the nutrient content were observed between the leaves of inoculated trees and the untreated trees. Mineral nutrient content in the leaves increased after mycorrhizal inoculation for N, P, K, Mn, Zn and decreased for Ca, B, Al and Cu. This study confirmed the efficacy of arbuscular mycorrhizal fungi to improve performance and salinity stress tolerance of citrus trees as host plant.

15:30 - 15:45

URBAN AGRICULTURE WITH AN EMPHASIS ON SOIL HEALTH, MYCORRHIZAL FUNGI AND COMMERCIAL MYCORRHIZAL INOCULANTS

M. Salomon¹, S. Watts-Williams², M. McLaughlin¹, T. Cavagnaro¹

¹*The Waite Research Institute and The School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, South Australia, Australia*

²*Australian Research Council Centre of Excellence in Plant Energy Biology, University of Adelaide, Adelaide, South Australia, Australia*

Urban agriculture provides a promising solution to improve food security and sustainability in food production, especially in light of future climate scenarios. Due to their surroundings, urban farms are highly influenced by anthropogenic interactions and, subsequently, urban soils need to be monitored for safety and their potential. This study includes 12 urban agriculture sites in the metropolitan area of Adelaide, Australia. The soil health analyses involved quantifying the concentrations of seven heavy metals, total and plant-available (Colwell) phosphorus, as well as total and available nitrogen. Presence of arbuscular mycorrhizal fungi was determined by means of spore extraction and a glasshouse bioassay. Additional inoculation with *Rhizophagus irregularis* provided information on the overall mycorrhizal potential of the soils. Subsequently, 10 commercial arbuscular mycorrhizal inocula were tested for their potential to colonise under laboratory and greenhouse conditions. All soil heavy metal concentrations were below Australian guideline levels for all tested samples, but there were differences between sites with or without an industrial history. Soils were adequately supplied with nitrogen (median = 0.7% total nitrogen) and although plant-available (Colwell) phosphorus was high (median = 442 mg kg⁻¹ soil), arbuscular mycorrhizal fungal spores were abundant. The glasshouse bioassay showed high mycorrhizal root colonization that was further increased for most soils after inoculation with *Rhizophagus irregularis*. While the commercial inoculants had a significant impact on plant growth, impacts on colonization were less clear.

It can be concluded that concerns over soil contamination in urban agriculture soils should be investigated in light of previous land use. Nutrient management with a preference for balanced fertilizers forms an important part of urban agriculture systems for the purpose of avoiding excess nutrient supply. Mycorrhizal root colonization could be increased by adding AMF inoculum to the garden beds; the effectivity of commercial inocula is unclear.

15:45 - 16:00

SOIL AMENDMENTS AFFECT MYCORRHIZAL COLONIZATION, ROOT NODULATION AND DRY MATTER ACCUMULATION IN COWPEA

IM Uzoh^{1,2}, KC Ramadile¹, UP Chukwudi^{3,4}, NO Igiehon¹, OO Babalola¹

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Cowpea is a proteinaceous plant used in combating malnutrition in Africa. Its protein content stems from symbiotic relationship with bacteria in its root nodule that fixes nitrogen. This study assessed the impact of soil amendment on the growth and yield of cowpea in 2017 and 2018. The experimental design was 3x2 factorial in randomized complete block design with three replications. Inorganic fertilizer, cattle dung and nutrient source control were applied to plots with and without biochar amendment. Data were collected on growth parameters, number of nodules, mycorrhizal colonization and plant dry matter accumulation. The results revealed that biochar amendment improved plant height by 8.8%, number of nodules by 14%, root length by 9.5%, root weight by 5.2% and dry matter accumulation by 92%. Application of NPK improved the root length and weight over the control. Stereomicroscopic visualization of the roots indicated high presence of arbuscules in the biochar treatment over the control. Biochar addition is essential for improvement of rhizosphere traits essential for plant growth enhancement.

16:00 - 16:15

THE INDUCTION OF NITRATE UPTAKE IN MAIZE PLANTS IS COUNTERACTED BY AZOSPIRILLUM BRASILENSE INOCULATION

Y. Pii¹, A. Aldrighetti¹, F. Valentinuzzi¹, T. Mimmo¹, S. Cesco¹

¹Free University of Bozen-Bolzano, Bolzano, Italia

Nitrogen (N) represents one of the main limiting factors for crop growth and productivity and, to date, it is widely supplied via application of fertilizers. However, the nutrient use efficiency of crops results rather limited, thus causing substantial losses of fertilizers. Plant nutrition might be improved by the use of plant growth-promoting rhizobacteria (PGPR). To this regard, the PGPR *Azospirillum brasilense* was already shown to be effective in enhancing plant growth and productivity, also influencing the quality of agricultural products. Furthermore, its interaction with host plants could modulate the nutrient acquisition mechanisms at root level, thus inducing a higher accumulation of micronutrients, such as iron.

On the bases of these observations, the influence of *A. brasilense* on nitrate uptake in maize plants was studied, particularly focusing on the high affinity transport system (HATS). To this aim, *Zea mays* L. seedlings were either non-inoculated or inoculated with *Azospirillum* and induced with 500 µM nitrate.

Azospirillum inoculation reduced the nitrate uptake rate in induced plants, suggesting that the bacterium might negatively affect HATS, at least over a short period. Nevertheless, the modulation of *ZmNF-YA* and *ZmLBD37* expression suggest that *Azospirillum* influences the N balance in plants, likely by supplying plants with reduced N, *i.e.* NH₄⁺. This hypothesis is further corroborated by the estimation of total N, which increased in inoculated plants independently from the nitrate provision, and by the expression of ammonium transporter genes, which was enhanced in the presence of *Azospirillum*.

Overall, the results hereby presented show that *A. brasilense* can counteract the plants' response to nitrate induction, albeit without compromising N nutrition. This evidence can be of particular relevance for the

agricultural practices, since the application of bioinoculants might require a fine-tuning of fertilizer inputs in order to avoid environmental and economic impacts.

16:15 - 16:30

PROTIST INTRODUCTION ENHANCES PLANT GROWTH BY RESHAPING THE RHIZOSPHERE MICROBIOME

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The microbiome is recognized as a major driver of plant growth, but harnessing its beneficial functions remains a challenge. Several attempts have been made to introduce beneficial microorganisms, but such approaches are often constrained by the low survival and efficiency of the introduced species. We therefore propose an alternative approach: instead of introducing plant-beneficial organisms, bacterivorous protists are applied to modulate the resident microbiome so as to promote bacteria carrying specific sets of plant beneficial traits. Free-living, heterotrophic protists are major consumers of bacteria in the rhizosphere and provide a competitive edge to species harboring a range of plant-beneficial traits. We therefore expected that introducing bacterivorous protists into the rhizosphere may be a powerful leverage to promote microbiome ability to stimulate plant growth.

We added six different protists species independently to soil containing a predator-free semi-natural bacterial community. After ten days of incubation, lettuce seedlings were transplanted to the resulting soils and plant performance tracked over time. Protist application modified the bacterial community in a species-dependent manner. Protist amendment slowed initial plant growth, but then lead to a rapid increase plant growth and nutritional value. This plant-beneficial effect was particularly strong at low nutrient availability. These effects were linked to an increase of plant-beneficial genes in the microbiome and enhanced microbial loop.

We propose that protists may be used as an inoculant to promote sustainable plant growth while minimizing fertilizer input.

16:30 - 16:45

ACCUMULATION OF MINERAL ELEMENTS IN THE RHIZOSPHERE AND CHICKPEA LEAVES: RESPONSE TO PHOSPHORUS AND RHIZOBIUM INOCULATION

V Madzivhandila¹, **JB Ogola**¹, ST Maseko²

¹*Department of Plant Production, University of Venda, Limpopo, South Africa*

²*Department of Crop Science, Gauteng, South Africa*

Legumes increase the solubilisation and accumulation of mineral nutrients in the rhizosphere and plant shoots but this depends on factors such as crop species, soil fertility, etc. We assessed the effect of fertiliser phosphorus and rhizobium inoculation on the concentration of nutrients in the rhizosphere and leaves of 2 chickpea genotypes. Field experiments were established at Thohoyandou and Syferkuil, South Africa in 2017. At both locations, accession 5 accumulated higher concentrations of nitrogen, phosphorus and potassium in

the rhizosphere compared to accession 1. Potassium in the rhizosphere was increased by rhizobium inoculation at Thohoyandou, and rhizosphere phosphorus was increased by the application of fertiliser phosphorus plus rhizobium at both locations. There was an increase in magnesium in the rhizosphere of chickpea grown with rhizobium inoculation plus the application of phosphorus at Thohoyandou. Nitrogen, phosphorus, potassium and calcium were markedly higher in leaves of accession 5 compared to accession 1 at both sites. Application of phosphorus, and rhizobium inoculant plus phosphorus resulted in greater accumulation of phosphorus at Thohoyandou. In contrast, zinc was significantly greater in leaves of inoculated chickpea plants at Syferkuil. Fertiliser phosphorus application markedly enhanced the accumulation of potassium in leaves of chickpea at Thohoyandou. At both locations, accession 5 exhibited greater ability for the uptake and accumulation of nutrients compared to accession 1. Furthermore, application of phosphorus alone and in combination with rhizobium inoculation increased the concentration of majority of the nutrients in the rhizosphere and plant tissue. Also, greater accumulation of nutrients in the rhizosphere, especially P and K, was associated with increased nutrient concentration in leaves. Clearly, fertiliser phosphorus and rhizobium inoculation are important for enhanced nutrient uptake and accumulation by chickpea but this may vary with genotypes.

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|----------------------|-----------------------|------------------------------------|
| 16:45 - 18:00 | POSTER SESSION | (Foyer & Salon A, D, E) |
| 19:00 - 23:00 | GALA DINNER | (CENTENNIAL HALL) |

08:30 - 09:30 KEYNOTE ADDRESS - MARCEL VAN DER HEIJDEN (SALON B+C)

ROOT MICROBIOME MANAGEMENT AND SOIL ECOLOGICAL ENGINEERING FOR A SUSTAINABLE AGRICULTURE

M van der Heijden²

¹*Agroscope, Zurich, Switzerland*

²*University of Zurich, Zurich, Switzerland*

Microbes represent the unseen majority of life on Earth. A handful of soil contains billions of bacterial cells, kilometers of fungal hyphae and a significant fraction of life's genetic diversity. Moreover, plant roots are colonized by diverse assemblages of fungi and prokaryotes and harbor a complex and well developed root microbiome. Recent work, including our own, highlights that ecosystem type, land use intensity and agricultural management (e.g. fertilisation and pesticide use) has a big impact on the composition of the soil, rhizosphere and root microbiome. For instance, we observed that plant roots in organically managed fields harbor highly complex microbial networks with a number of keystone taxa, while roots of conventionally managed fields contain less developed microbial communities with simple microbial networks. So far, it is still poorly understood how changes in the soil and root microbiome influence plant growth and ecosystem functioning. Our work reveals that soil and root microbial diversity can have a positive influence on plant growth and a number of important ecosystem functions including plant diversity, nutrient acquisition, nutrient cycling and decomposition. In addition, our newest results indicate that microbiome complexity and interkingdom associations between various groups of microbes have a big impact on microbiome performance and influence plant performance and ecosystem functions. Field inoculation trials with mycorrhizal fungi and other biologicals are underway to test whether it is possible to improve plant productivity and complement the microbiome by adding microbes that are absent or low in abundance. Finally, I will highlight avenues for further research and discuss how soil ecological engineering and microbiome management can be used as tools to enhance ecosystem sustainability and sustainable food production.

09:30 - 10:00 POSTER PITCHES (SALON B+C)

10:00 - 10:30 HEALTH BREAK & POSTERS & EXHIBITS (FOYER & SALON A, D, E)

SESSION 13: PLANT HOLOBIONT

(GALLERY A)

Session Chair: Angela Sessitsch

10:30 - 11:00

SEEDS - RESERVOIR AND VECTOR OF DIVERSE AND FUNCTIONALLY IMPORTANT MICROBIOTA

A. Sessitsch¹, C. Escobar Rodriguez¹, S. Compant¹, B. Mitter¹¹AIT Austrian Institute of Technology, Tulln, Austria

The plant holobiont comprises highly diverse microbiomes, which complement plant traits and contribute to plant growth and health. Plants provide different micro-environments for microbial colonization such as the rhizosphere, phyllosphere or endosphere and different plant tissues and microbiome diversity depends on the habitat, plant growth stage, plant genotype and environmental conditions. Endophytes, which live at least part of their life cycle within plants, are particularly interesting as they have an intimate relationship with their host. Many endophytes derive from the soil / rhizosphere environment and may systematically colonize other plant tissues such as stems, leaves and even reproductive organs. Working with different plant species such as potato, maize, wheat and the model plant *Setaria viridis*, we have found highly tissue-specific microbial communities indicating high sensitivity and response to the distinct physiological conditions encountered in different plant tissues. Of particular interest are seed endophytes. They show rather low complexity but represent important members of the next generation plant and may be highly important for early plant establishment. By analysing microbiomes associated with different tissues, particularly seeds, of *Setaria viridis* and *S. pumila* we revealed information regarding the ecology and functioning of seed-associated microbiota. Seeds represent also an important vector for the transmission of microbiota influencing the plant performance of the next generation of plants and maybe used as a vector to deliver microbial inoculants into target crop plants.

11:00 - 11:15

HAS AGRICULTURAL INTENSIFICATION CAUSED HOLOBIONT-LEVEL ADAPTATION IN MAIZE?

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Agricultural intensification, particularly the introduction of synthetic nitrogen (N) fertilizer, has fundamentally altered maize agroecosystems in the United States. Significantly, the dominant form of N supplied to meet crop demand has changed: inputs of organic matter from cover crops and compost have declined in favor of inorganic N fertilizers. Selective pressures on maize roots and rhizosphere interactions have consequently shifted, as mineralization and N-cycling processes mediated by rhizosphere microbes are less important when N is supplied in forms readily available for plant uptake. Previous studies have shown effects of plant breeding

on maize root architecture and N uptake but have not investigated how plant-microbe interactions in the rhizosphere may have responded to the changing agricultural environment. We used stable isotope labeling in a rhizobox experiment comparing six maize genotypes released before or after the introduction of synthetic fertilizer in the United States to determine whether adaptation to different N forms has occurred at the holobiont level. We hypothesized that holobiont-level adaptation has resulted in improved root plasticity and upregulation of denitrification-related microbial gene expression (*nirS*, *nirK*, *nosZ*) in response to inorganic N but decreased root plasticity, extracellular enzyme activity (urease, β -glucosidase, N-acetyl-glucosaminidase, leucyl aminopeptidase), and microbial N mineralization gene expression (*apr*, *npr*) in response to organic N. Genotypic differences in enzyme activity but not root plasticity or microbial N-cycling gene expression underscore the complex nature of rhizosphere interactions and highlight the need for further research into the mechanisms underlying holobiont-level adaptation.

11:15 - 11:30

NITROGEN AVAILABILITY MODULATES THE HOST CONTROL OF THE BARLEY RHIZOSPHERE MICROBIOTA

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Since the dawn of agriculture, human selection on plants has progressively differentiated input-demanding crops from their wild progenitors thriving in marginal areas. Barley (*Hordeum vulgare*) is a prime example of this process. We previously demonstrated that wild and domesticated barley genotypes host distinct microbial communities in their rhizosphere. Here, we tested the hypothesis that microbiome diversification is modulated by nutrient availability in soil and we assessed the contribution of the barley microbiome to plant growth under nutrient sub-optimal conditions.

We grew two wild (*H. vulgare ssp. spontaneum*) and a domesticated (*H. vulgare ssp. vulgare*) barley genotypes in an agricultural soil amended with limiting, sub-optimal and sufficient nitrogen (N) supplies. We determined the impact of these treatments on soil and plant N as well as on stem dry weight, a proxy for plant growth. By using a two-pronged 16S rRNA gene survey and a shotgun metagenomics approach, we determined the impact of N availability on the taxonomic and functional composition of the microbiome inhabiting the barley rhizosphere and unplanted soil controls. A plant-soil feedback experiment was implemented to assess the

contribution of the wild and domesticated rhizosphere microbiome to plant growth under sub-optimal substrate conditions.

Domesticated and wild genotypes displayed contrasting responsiveness to N amendments, likely reflecting a footprint of their differential evolutionary histories. Rhizosphere profiles were distinct from bulk soil controls and displayed a significant N-dependent taxonomic diversification which is maximised under limited N conditions. Strikingly, this diversification mirrors a metabolic specialisation of the barley microbiome, with a bias for functions implicated in nitrogen and sulphur metabolism in a wild genotype as opposed to the RNA and cell wall metabolisms in the domesticated genotype. Congruently, the plant-soil feedback experiment indicated a limited yet significant probiotic effect of the wild barley microbiome.

11:30 - 11:45

ROLE OF N-ACYL-HOMOSERINE LACTONE QUORUM SENSING COMPOUNDS OF GRAM-NEGATIVE BACTERIA FOR BENEFICIAL HOLOBIONTIC MICROBE-PLANT INTERACTIONS

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Beneficial endophytic bacterial interactions in holobiontic plant systems depend on specific signals. Compared to well-studied symbiotic legume systems, much less is known for the interaction of endophytic bacteria with non-legume crop plants. The objective of our studies was to elucidate the involvement of *N*-acyl-homoserine lactones (AHLs), the quorum sensing signals of Gram-negative bacteria.

Many Gram-negative plant growth promoting bacteria are able to produce several types of AHLs with hydrocarbon chains from 4 to 14 C-atoms. When pure C8- or C12-AHL compounds were applied to roots of barley seedlings, morphological and physiological changes occur in the whole plant. In addition, priming of systemic plant defense responses was recorded, which was accompanied by increased levels of salicylic acid and nitric oxide (NO) in plant tissues. Thus, AHL compounds of beneficial rhizobacteria are candidates to play an important role in endophytic plant colonization and improved plant performance. The colonization patterns of AHL-producing and AHL-defective mutants of PGPR-bacteria were examined by confocal laser scanning microscopy using fluorescently tagged bacteria and gene expression profiles of bacteria and plants with RNA seq and quantitative RT-PCR. Wild type and synthase/receptor-mutants (unable to synthesize or sense AHLs) of the diazotrophic endophyte *Gluconacetobacter diazotrophicus* PAL5, and *Acidovorax radialis* N35, a beneficial endophyte of wheat and barley, were studied. A GFP-labelled AHL receptor mutant of *G. diazotrophicus* PAL5 was unable to colonize rice endophytically in contrast to the wild type. An AHL-synthase mutant of *A. radialis* N35 also showed less efficient endophytic colonization. Surprisingly, the expression profile of plant genes during colonization by the AHL-synthase mutant showed defense responses like the expression of flavonoid biosynthesis genes and increased flavonoid production, while the wild type caused only some priming responses. Therefore, AHLs probably modulates the expression of key bacterial proteins for productive interaction with plants and/or by inducing beneficial plant responses.

11:45 - 12:00

ARE THE CHANGES IN THE METABOLIC ACTIVITY OF THE ARBUSCULAR MYCORRHIZAL HYPHOSPHERE WARNING NEIGHBOURING PLANTS?

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Research has revealed that arbuscular mycorrhizal underground mycelial networks are used as ‘communication highways’, whereby warning signals are transported between neighbouring plants after a biotic or abiotic stress event. We hypothesised that a stress event applied to the phyllosphere of one plant would initiate a signalling event travelling through the arbuscular mycorrhizal hyphosphere that could alter the metabolic activity in the hyphosphere connecting plants.

Plants were directly challenged with either a pest or pathogen for 72 or 84 hours, whilst expression of relevant plant defence genes was evaluated in connected plants. The metabolic profiles, as well as changes in microbial biomass of the hyphosphere connecting plants were characterised in detail.

Induction of plant defence in the connected plants occurred 12 hours after stress application, which was independent of stress type, however, the magnitude of the response differed. The hexose levels in the hyphosphere increased after pest challenge, whilst mannitol levels decreased after a pathogen challenge. Conversely, whilst biomass of gram-positive bacteria increased after a pest challenge, the level of actinobacteria decreased after pathogen challenge. Therefore, the metabolic changes observed in both in the phyllosphere and in the hyphosphere of connected plants differed, depending on the combination of stressor, plant and arbuscular mycorrhizal fungus. Future work must therefore focus on the mechanistic side of these interactions, by evaluating different genotype combinations.

This work is the first to reveal metabolic profiles of the arbuscular mycorrhizal hyphosphere, as well as demonstrating the impact of stress events to both the phyllosphere and to changes in the activity of the arbuscular mycorrhizal hyphosphere due to signalling events occurring between plants connected by the arbuscular mycorrhizal hyphosphere.

12:00 - 12:15

THE PLANT MICROBIOME OF BRASSICA CARINATA AND ITS POTENTIAL TO INCREASE PLANT GROWTH AND YIELD

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Traditional agricultural practices use extensive amounts of manmade and artificial fertilizers. Fertilizer inputs while helpful for yield increase, are extremely costly for both the environment, but also for growers and consumers. The need for increasing crop yield production and understanding the connections between plants and microbes has never been greater. The plant microbiome and the design of a plant microbiome with specific capabilities could offer an attractive solution that could be included in current agricultural practices without the need for additional farming technology. *Brassica carinata*, an oilseed producing crop, was selected as a plant of interest because due to its high heat and drought tolerance, and the potential that the oil can be

developed into aviation biofuel for jet engines. To better understand the natural bacterial community of *Brassica carinata* and the effects of individual microbes on *B. carinata*, we extracted genomic DNA and processed the samples through a metataxonomic pipeline. At the same time, individual endophytic bacterial isolates were extracted and isolated on minimal nitrogen-free media to select for their capability to fix gaseous nitrogen. *In-vitro* growth promoting tests were also performed to screen the isolated bacteria for their plant growth promoting capabilities. Isolates were also applied to other crop species and different crop varieties in greenhouse and field trials to determine their effect on plant growth and yield under different stress environments. We will review our results on the plant microbiome of *Brassica carinata*, and will discuss the capability of isolated endophytes to promote the growth of a broad range of crops under different stress environments. We will also discuss the capability of the different endophytic strains to change the microbiome of corn plants in field experiments.

12:15 - 12:30

PLANT LIFE CYCLE AND ENVIRONMENTAL CONDITIONS SHAPE THE COMPOSITION OF FUNGAL MICROBIOME IN CANOLA GENOTYPES

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Canola (*Brassica napus* L.) is an economically important crop in Canada. The plant microbiome is key to plant health and crop productivity, but it is unknown to what extent the canola fungal root microbiome is modulated by plant genetic controls. Over ten consecutive weeks, we surveyed the root and rhizosphere microbiome of three replicates of eight genetically diverse canola genotypes in 2016 and 2017 grown near Saskatoon, SK. Single nucleotide variant (SNV) analysis of internal transcribed spacer (ITS) region amplicons revealed that canola genotype shapes the composition of canola root ($P=0.01$) and rhizosphere ($P=0.02$) fungal communities. Root fungal communities were dominated by a single species, *Olpidium brassicae*, comprising 61-81% of the fungal ITS libraries across all genotypes throughout the ten-week sampling period from vegetative to maturity stages. Year-to-year and week-to-week differences dominated changes of both the root and rhizosphere fungal communities ($P<0.001$). Canola genotype did not interact with sampling time at different phenological stages, but instead interacted with year ($P<0.001$) in shaping the composition of the rhizosphere and root fungal microbiome dynamics. Community structures were more dispersed in 2017 compared to 2016 when precipitation was more limiting for crop growth. Overall, the canola genotype, phenology and environmental factors determine the composition of fungal root microbiome. The genotypic differences indicate the potential for improving the ability of canola to exploit the soil fungal resources for promoting crop productivity.

Session Chair: *Melissa Arcand*

10:30 - 10:45

INFLUENCE OF ROOT HAIRS AND RHIZOSPHERE ACIDIFICATION ON PHOSPHORUS MOBILIZATION FROM ALKALINE SOILS

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Although the role of root hairs in the uptake of immobile elements such as phosphorous is well accepted, their importance in phosphorus mobilization from alkaline soil remain largely unknown. Furthermore, nutrient release in the rhizosphere can be enhanced by an increased release of organic anions or exoenzymes. To this end, we conducted a rhizobox experiment combining Phosphorus-33 Phosphor imaging with pH planar optodes to investigate the role of root hairs and their release of organic acids on phosphorus mobilization in the rhizosphere. Two varieties of maize and barley, with and without root hairs (wild type vs mutant), were tested for their potential to acidify the rhizosphere and increase phosphorus mobilization. The alkaline soil (pH 8.3) was homogenously labeled with Phosphorus-33-orthophosphate. Additionally, nitrogen fertilization was tested to explore the impact of nitrogen availability on phosphorus mobilization. We applied both imaging techniques at five time points to compare Phosphorus-33 distribution and progress of acidification. After 25 days, the plants were harvested and analyzed for Phosphorus-33.

Acidification occurred at younger root segments of maize and barley roots, indicating that an accumulation of organic acids is required until a decrease in pH could be observed.

While barley biomass production was mainly affected by nitrogen fertilization, maize showed a positive response to nitrogen and root hairs. However, root hairs had a major contribution to the amount of Phosphorus-33 in barley tissue, which was consistent with the depletion zone gained from the Phosphor imaging.

Maize had no differences in Phosphorus-33 amount between the treatments, whereas the depletion zone was mainly affected by nitrogen fertilization. These results indicate that barley and maize may have different strategies to mobilize phosphorus from alkaline soil. We assume that mycorrhization as well as the development of lateral roots are additional strategies to improve phosphorus mobilization.

10:45 - 11:00

PHOSPHORUS-ACQUISITION STRATEGIES OF NATIVE PLANT SPECIES OF CAMPO NATURAL GRASSLANDS OF SOUTHERN SOUTH AMERICA

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The role of plant functional traits associated with phosphorus acquisition in shaping the structure and functioning of plant communities is receiving increasing attention. Two biodiversity hotspots in the southern hemisphere have been studied, identifying the diversity in plant phosphorus-acquisition strategies and their links with soil properties. We focus on the diversity of plant phosphorus-acquisition traits of species from the Campo natural grasslands, to test the relationships between them and the effect of variation in soil attributes among communities. We sampled 110 plant species in seven herbaceous communities associated with different soil geomorphology, and quantified root arbuscular mycorrhizal colonization and extracellular root phosphomonoesterase and phosphodiesterase activity. Readily available soil phosphorus was closely associated with variation in phosphorus-acquisition strategy. Cyperaceae, Asteraceae and Poaceae, the most common families of these communities, had low levels of arbuscular mycorrhizal fungi colonization; 80 %, 53 % and 34 % of total observations, respectively, had arbuscular mycorrhizal colonization values below 2 %, while 92 % of the plant species had colonization levels below 10 %. Cyperaceae had the highest values of root phosphomonoesterase and phosphodiesterase activity (27.8 and 8.5 mg phosphorus g⁻¹ root hour⁻¹, respectively), followed by Poaceae (25.3 and 6.0 mg phosphorus g⁻¹ root hour⁻¹ respectively) and Asteraceae (15.5 and 6.0 mg phosphorus g⁻¹ root hour⁻¹ respectively). Community-weighted values of arbuscular mycorrhizal fungi colonization increased with increasing readily-available soil phosphorus. The opposite was found for community-weighted root phosphomonoesterase and phosphodiesterase activity values. Notably, these community-level responses were not observed at an individual species level. These results suggest that soil phosphorus availability strongly modulates the dominant phosphorus-acquisition strategies, and thus plant functioning, in species-rich Campo natural grasslands of South America.

11:00 - 11:15

ARBUSCULAR MYCORRHIZAL FUNGI SECRETE ACID PHOSPHATASE TO HYPHOSPHERE IN RESPONSE TO PHOSPHORUS DEFICIENCY

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Arbuscular mycorrhizal (AM) fungi increase phosphorus (P) uptake in plants. Some plants can utilize organic P by secreting acid phosphatase (ACP) from the roots, especially under low P conditions. Recently, the secretion of ACP from extraradical hyphae of AM fungi has been reported. However, environmental factors that promote the secretion of ACP are unknown. The objective of the present study was to investigate whether the secretion of ACP from extraradical hyphae is induced by low P conditions. *Allium fistulosum* were either inoculated with the AM fungus *Rhizophagus clarus* strain CK001 or remained uninoculated and were grown in soil with or without 0.5 g P₂O₅ kg soil⁻¹ of two-compartment pots. Soil solution was collected 45 days after sowing and was analyzed for ACP activity. Ri T-DNA transformed roots of *Linum usitatissimum* inoculated with *R. clarus* were grown with 3 and 30 μM KH₂PO₄ using two-compartment Petri dishes. Hyphal exudates, extraradical hyphae, and hairy roots were collected and analyzed for ACP activity. In the pot experiment ACP activity in the soil solution of the hyphal compartment in the inoculation treatment was higher in those specimens without P fertilization than in those with P fertilization 45 days after sowing. In the in vitro two-compartment culture, ACP activity of hyphal exudates and extraradical hyphae were higher in those under the 3 μM treatment than in those under the 30 μM treatment. These findings suggest that the secretion of ACP from the extraradical hyphae of *R. clarus* into the hyphosphere is promoted under low P conditions.

11:15 - 11:30

MODELING THE COMPARATIVE IMPACT OF ROOT HAIRS ON PHOSPHORUS UPTAKE UNDER DIFFERENT FIELD CONDITIONS

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Root hairs play a significant role in Phosphorous (P) extraction at the pore scale. However, their importance at the field scale remains poorly understood. This study uses a continuum model to explore the relative impact that root hairs have on the large-scale uptake of P, comparing root hair influence under different agricultural treatments. Poor vs rich soil P stocks are compared for uniform vs decaying P distributions down the soil profile along with early vs late precipitation scenarios. All of the simulation cases are run assessing the relative impact of root hairs vs scenarios without root hairs. Simulation results suggest root hairs accounted for 50% of total P

uptake by plants. Furthermore, results also show that late initiation time of precipitation played a crucial role in P uptake, potentially limiting the uptake rate by over 60% depending on the growth period. Despite the large differences in the uptake rate, changes in the soil P concentration in the domain due to root solute uptake remains marginal when considering a single growth season. However, over the duration of six years, simulation results showed that noticeable differences arise for realistic precipitation events. Results suggest that due to the low mobility of P, differences in the profiles are marginal. However, our simulations show that root P uptake efficiency could be enhanced by coordinating irrigation/precipitation with P application during earlier growth stages of crops.

11:30 - 11:45

MONITORING PHOSPHORUS MOBILITY IN SOIL RELEVANT FOR ROOT UPTAKE USING MICRODIALYSIS AND X-RAY COMPUTED TOMOGRAPHY

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Phosphorus availability is a limiting factor for plant growth, and plant uptake strongly depends on its speciation and chemical interactions within the soil. Phosphorus diffusion in soil is very slow and it is influenced by both abiotic, like dissolution and adsorption, and biological processes. Plants have developed strategies to enhance phosphate uptake by optimizing root architecture and exuding compounds that can help in mobilizing phosphorus from the surrounding soil.

Microdialysis is a powerful technique for monitoring nutrients diffusion in soil because it allows for time resolved samples *in-situ* without disrupting the soil structure.

In this work, an experimental assay for monitoring phosphorus diffusion from a fertilizer pellet and its mobility in soil using microdialysis probes in a soil column was designed. Preliminary tests have been conducted in a mesocosm system in order to assess spatial and temporal scales for phosphorus diffusion in a low-mobility phosphorus soil.

X-ray computed tomography was then used to visualize the position of the microdialysis probes and the pellet in the soil column and it will be employed in further assays to determine the position of the probes relative to the roots of a rice (*Oryza sativa*) plant. The combination of X-ray computed tomography and microdialysis will be used to measure the impact of roots on phosphorus mobility.

11:45 - 12:00

WHAT IS THE ACTUAL MERIT OF CLUSTER ROOT FORMATION IN PHOSPHORUS UPTAKE FROM REAL SOIL?

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A unique plant group, such as the family Proteaceae and white lupin (*Lupinus albus*), develops cluster roots (CRs) under phosphorus (P)-deficient conditions. Previous physiological studies revealed that CRs release P-mobilizing substances, such as carboxylates and acid phosphatases. However, it is still unknown whether the root exudates from CRs substantially contribute to P uptake from the dynamic P pool in rhizosphere soil. This study aimed to clarify whether the P-mobilizing capacity of CRs contributes to actual P uptake from soil.

P dynamics of the rhizosphere soil of CRs formed by *Helicia cochinchinensis*, which is a Japanese native Proteaceae, and white lupin were analyzed by sequential fractionation. The amount of total P in the rhizosphere soils was dramatically decreased. Sparingly-soluble organic-P fractions were most abundant among the decreased P fractions in both species. Physiological evidence of high exudation of citrate and high activities of phosphatase in the rhizosphere of CRs supported this result. Our results suggest that the sparingly-soluble organic-P fraction was solubilized by chelation of carboxylates and subsequently hydrolyzed by phosphatase in the CR rhizosphere.

Root exudates were collected from white lupin and narrow-leaf lupin (*Lupinus angustifolius*) grown under -P condition. Citrate and phosphatase activity in whole root exudates were higher in narrow-leaf lupin than in white lupin, but narrow-leaf lupin never formed any CRs. However, the amount of P uptake per plant was significantly greater in white lupin than in narrow-leaf lupin grown in a low-P soil. Mobilization of sparingly-soluble organic-P was greater in the rhizosphere of white lupin CRs than in non-cluster roots of narrow-leaf lupin. We conclude that an important advantage of CR formation is the spatial strategy to mobilize sparingly available P, which is unevenly distributed in soils.

12:00 - 12:15

THE WHEAT ROOT SECRETED PROTEOME IN THE CONTEXT OF PLANT PHOSPHORUS NUTRITION

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Plant roots release a diverse range of small molecules and polymeric substances, including proteins, capable of interacting with the local soil environment. Proteins that accumulate in extracellular spaces are promising targets in breeding crops for enhanced performance on soils characterised by low phosphorus availability. A highly annotated wheat genome has been released recently and the expressed wheat proteome has been experimentally analysed inside various tissue types and between developmental stages. However, a comprehensive map of the proteins released by wheat roots still remains to be established in order to characterise their functional potential in rhizosphere processes and to provide the foundation for high-throughput analytics such as multiple reaction monitoring. For this purpose, we collected mucilage exuded by the tips of wheat seedling roots and analysed its proteome via untargeted liquid chromatography – tandem mass spectrometry. More than 2100 proteins were identified in the root tips and 335 in root mucilage, respectively. Relative to the root tip intracellular proteome, the proteome present in this highly viscous extracellular environment is characterized by a high number of stress, defence, protein processing and cell wall biosynthesis-related proteins. An analysis of predicted cellular targeting revealed a significant enrichment of

proteins destined for the apoplast and extracellular spaces. This qualitative study represents the basis for the future targeted quantification of wheat extracellular proteins in order to understand spatial and time-resolved phosphorus dynamics in the rhizosphere.

12:15 - 12:30

BIOGEOCHEMICAL PHENOTYPING OF WINTER WHEAT FOR PHOSPHORUS ACQUISITION

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Important to the Green Revolution was the development of semi-dwarf and higher yielding wheat varieties that were more resistant to lodging. Norin-10, a dwarf wheat variety from Japan, was introduced into US breeding lines during the 1950s to develop semi-dwarf lines. Development of these new lines typically occurred in fertilized conditions and focused solely on aboveground traits, such as shoot length, grain yield, and disease resistance. As a result, belowground traits related to nutrient acquisition may have been lost over time. This study assessed both root system architecture and chemical traits in a diverse panel of winter wheat lines. Cultivars were primarily selected based on decade of release and were further selected based on genotypic coancestry. A panel of ten cultivars were grown in gel and imaged using light tomography to create three-dimensional images of root systems during growth. Root system architectural traits including seminal root angle, bushiness, network area, maximum vertical depth, and root number were determined among other parameters using GiA Roots software. Cultivars were also grown in liquid culture under phosphorus limiting conditions for analysis of chemical traits including fresh root organic acid concentration, exudate composition, and root quality. Phosphorus solubilizing potential was calculated using both RSA and chemical traits to identify cultivars with low or high phosphorus-scavenging potential.

SESSION 15: CUTTING-EDGE APPROACHES AND RHIZOSPHERE MODELLING (GALLERY C)

Session Chair: Lori Phillips

10:30 - 10:45

WHAT DID WE LEARN IN 58 YEARS OF RHIZOSPHERE MODELING AND WHERE TO GO NEXT

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The rhizosphere is the contact zone between root and soil. It provides important nutrients to the plant, and has complex soil chemistry and biology that is distinct to the bulk soil. Many rhizosphere models have been developed to describe the complex relations and associated dynamic processes influencing nutrient availability and root nutrient uptake. The first rhizosphere models were developed in the early 1960s and described transport of phosphorus in soil solution through the rhizosphere towards the root. These models considered diffusion and linear adsorption around a radial or cylindrical root. They described the transport and uptake but

lacked biological interactions by microorganisms. Later models included advection, rapid or slow sorption, distinguishing the soil liquid and solid phase, and different uptake terms for root surface and root hairs. This led to a family of classical models that share a common structure of mathematically realized processes, but include many variations thereof. Thus different rhizosphere components, including various nutrients, metals, and microbes can be modeled.

These rhizosphere components have been coupled over functional relations of solubility, sorption, and reactions in the transport equation. Such coupled models have been used to answer: What mechanisms influence nutrient availability and uptake, and how does the plant phenotype influence nutrient uptake? One of the most challenging aspects of these models remains their experimental validation and parameterization for various realistic environments. This becomes even more challenging as the number of coupled components increases. Current models are nevertheless still relatively simple compared to the complexity of the rhizosphere. Upscaling to the whole root system is often done in a simplified way over root length density functions, but we suggest could be better done using root architectural models. We will present a review of key models used for rhizosphere processes and how these models might be coupled.

10:45 - 11:00

MODELING THE IMPACT OF BIOPORES ON ROOT GROWTH AND ROOT WATER UPTAKE UNDER DIFFERENT SOIL PHYSICAL AND ENVIRONMENTAL CONDITIONS

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Roots are known to use biopores as preferential growth pathways to overcome hard soil layers and access subsoil water and nutrient resources. This study evaluates root-biopore interactions at the root-system scale under different soil physical and environmental conditions using a mechanistic simulation model and extensive experimental field data. In the field experiment, spring wheat was grown on silt loam with a large biopore density. Microcomputed tomography scans of large soil columns from the field site were used to provide a realistic biopore network as input for our three-dimensional numerical soil-root-interaction model, which was then applied to simulate root architecture as well as water flow in the root-biopore-soil continuum. The model was calibrated against observed root length densities in both bulk soil and biopores by optimizing root growth model input parameters. By implementing known interactions between root growth and soil penetration resistance into our model, we could simulate root systems whose response to biopores corresponded well to both our field experimental observations and to descriptions from literature such as increased total root length and increased rooting depth. For all considered soil physical (soil texture and bulk density) and environmental (years of varying dryness) conditions, we found biopores to substantially mitigate transpiration deficits in times of drought by allowing roots to take up water from wetter and deeper soil layers. This was even the case when

assuming reduced root water uptake in biopores due to limited root-soil contact. The beneficial impact of biopores on root water uptake was larger for more compact soil and for soil that was less conductive at low soil water potentials.

11:00 - 11:15

IMAGING OF ROOTS AND PORE NETWORKS IN SOIL SYSTEMS BY USING HIGH RESOLUTION X-RAY MICRO-CT

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Today's high-resolution X-ray CT with its powerful tubes and great detail detectability lends itself naturally to geological and pedological applications. Those include the non-destructive interior examination and textural analysis of rock and soil samples and their permeability and porosity – to name only a few. Especially spatial distribution and geometry of pores, mineral phases and fractures are important for the evaluation of hydrologic and aeration properties in soils as well as for root development in the soil matrix. The possibility to visualize a whole soil aggregate or root tissue in a non-destructive way is undoubtedly the most valuable feature of this type of analysis and is a new area for routine application of high resolution X-ray micro-CT.

We will address visualization and quantification of porous networks in 3D in different environmental samples ranging from clastic sedimentary rock to soil cores and individual soil aggregates. As several processes and habitat functions are related to various pore sizes imaging of the intact soil matrix will be presented on different scales of interest – from the mm-scale representing the connectivity of macro-pores down to the micro-scale representing the space of microbial habitats. Therefore, soils were impregnated with resin and scanned via X-ray CT with the phoenix nanotom m. Scans at higher resolution were obtained from sub-volumes cut from the entire resin impregnated block and from crop roots surrounded by rhizosphere soil. Within the scanned structures we will highlight interfaces i.e. pore-solid interface and soil-root interface. The latter will be linked to examples of fluorescent microscopy and scanning electron microscopy obtained from 2D sections revealing additional biological and chemical information in the respective micro-environment. Based on the combination of all 3D and 2D imaging data habitat features of soils can be characterized and combined with studies analyzing microbial rhizosphere colonization.

11:15 - 11:30

CAPTURING SOLUTE PENETRATION THROUGH RIDGE AND FURROW OR FLAT PLANTING SYSTEMS USING X-RAY COMPUTED TOMOGRAPHY

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Two planting systems widely used within the United Kingdom for arable cultivation are 'flat planting' and 'ridge/furrow planting'. Flat planting uses an even soil surface with no significant spatial geometric changes whereas ridge/furrow planting involves the creation of a system of periodic peaks and troughs. It has been shown that the mechanisms of water movement within ridge/furrow systems could make them more vulnerable to solute leaching, for example, during the application of soluble agrochemicals. Previous field scale agronomic studies demonstrated solutes within the furrows penetrate to greater absolute depths in all instances. Mathematical modelling studies indicate that a ridge/furrow system could either increase or reduce the penetration depth of solutes, relative to flat systems, often depending on the rainfall activity following solute application. For example, when solute application to a ridge/furrow system was followed by little rainfall, the penetration depth of solutes was reduced compared to flat systems. However, when considerably more rainfall followed the application of the solute, the penetration depth of the solute was increased in ridge/furrow systems compared to flat.

We experimentally observed solute movement through ridge/furrow and flat soil systems using time-resolved X-ray computed tomography imaging. We traced the movement and distribution patterns of a soluble iodinated contrast media through soil columns. Experimental treatments included ridge/furrow or flat soil structure, the presence of surface water ponding following the application of the contrast media and the inclusion of rice plants. Experimental image results were compared to numerical simulations, based upon existing models. Similar penetration patterns were observed in experimental and numerical results. The results demonstrate that plant roots, soil surface structure and soil moisture can significantly affect the penetration depth of solutes.

11:30 - 11:45

TRACE GASES: EXTENDING THE LIMITS OF RHIZOSPHERE

A. de la Porte¹, E. Yergeau¹, P. Constant¹

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Legume plants are involved in a mutualistic relationship with soilborne bacteria, known as rhizobia, fixing atmospheric nitrogen (N_2) in legumes' symbiotic organs, nodules. Legumes that host rhizobia lacking the hydrogenase uptake system release hydrogen (H_2) from their nodules as a by-product of N_2 fixation. The high H_2 concentration area around nodules could radiated up to 10cm, as determined by modelling, thus drastically extending the classical limits of few millimeters generally considered as the rhizosphere. H_2 is an energetic compound that is readily consumed by soil H_2 -oxidising microbes, thus potentially impacting rhizospheric community functioning. For instance, it has been shown that H_2 displays a soil fertilisation effect, but the underlying processes remain unknown. Our aim is to test the hypothesis that H_2 fertilisation effect is driven by

changes in microbial-mediated processes related to nutrient turnover. Our objectives are (1) to record H₂ diffusion from nodules in the rhizosphere and its concentration gradients, as measured by gas chromatography, and (2) identify key microbial guilds whose biodiversity and activity is impaired by changes in H₂ concentrations. For the purpose of these experiments, we developed an innovative rhizotron system designed to collect gas samples in soil and examine spatial correlations between H₂ gradients and changes in microbial functions. Here, we present performance of rhizotron system to monitor trace gas diffusion and microbial turnover in soil. Case studies including soil alone, soil exposed to H₂ originating from artificial or legume plants are presented. Taken together, the results demonstrate that non-destructive and non-disturbing gas sampling in the rhizotron system permits temporal studies mimicking *in situ* conditions to study the impact of gas emission from plants on soil microbial community diversity and functioning.

11:45 - 12:00

NEW STRATEGIES FOR ASSESSING MICROBIAL INTERACTIONS IN THE RHIZOSPHERE

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Microbiomes are understood to be important drivers of plant and soil health. However, the large variability in these environments makes them challenging to study in a reproducible manner, particularly under defined, yet environmentally relevant conditions. To address this need, we recently developed and published methods for making and using fabricated, single plant-scale ecosystems (EcoFABs) for the research of plant and soil microbiomes (www.eco-fab.org; <https://doi.org/10.3791/57170>). EcoFABs contain root chambers (1.5 – 12 ml) for imaging root phenotypes and microbial colonization, allow for sampling of plant exudates and have been tested with a number of plant species and growth matrices.

To assess the reproducibility of plant and metabolite measurements within EcoFABs, a ring-trial was conducted between four laboratories growing the model plant *Brachypodium distachyon* within EcoFABs. We are now expanding the capabilities of EcoFABs and examining methods for disseminating them to the scientific community. This includes the generation of microbiomes for use within EcoFABs and the development of an automated system for the incubation, sampling and imaging of EcoFABs at Lawrence Berkeley National Laboratory.

Results/Conclusions: The ring-trial revealed that effects were statistically different between treatments, but not researchers, both with respect to plant physiology and metabolic traits. Interestingly, the addition of a cell-free soil extract caused a significant increase in root hair length along with indications that the plants took up a significant portion (~50%) of the compounds in the extract (<https://doi.org/10.1111/nph.15662>).

Our initial microbiome is composed of (>20) bacteria isolated from switchgrass rhizospheres. These bacteria, being sequenced and metabolically profiled, were selected based on several criteria, including: relative abundance in field surveys, enrichment in the rhizosphere, in-situ activity and reproducible growth in

communities. The automated system being built will enable large ecological studies by users that may design experiments with a selectable microbial community, growth conditions and media/substrate concentrations.

12:00 - 12:15

TRACKING 3D WATER FLOW AND ROOT UPTAKE IN SOIL BY ULTRA-FAST NEUTRON TOMOGRAPHY

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Neutron imaging is a non-invasive technique providing experimental access to the rhizosphere hydraulics. Neutrons penetrate deeply into soil minerals while being very sensitive for water. For this reason, they can precisely sense position and shape of roots (with water being the main constituent) along with the distribution of water in the surrounding soil. Taking advantage of this detection capability 2D neutron radiography is increasingly being used to analyze dynamic hydraulic interaction between roots and soil. However, with respect to the usual complexity of the 3D root system architecture the analysis of water transfer processes in the rhizosphere calls for tomographic (3D) imaging approaches. Until recently, the usual acquisition time for a neutron tomogram was about one hour or even longer, which restricted the applicability of neutron tomography to the observation of root systems under quasi-stationary hydraulic conditions, thus, excluding dynamic events such as tracer infiltration and subsequent root uptake. A substantial acceleration of the acquisition process was necessary to resolve the water transfer through the rhizosphere in three dimensions. Employing a new exposure mode, we drastically reduced the acquisition time for a neutron tomogram to 10 s. This technical breakthrough paving the way for dynamic studies of 3D flow processes in porous media. We tested and adjusted the novel imaging approach in a series of D₂O tracer experiments with young potted lupine and maize plants. We succeeded to visualize the water infiltration of soil and to track and analyze the subsequent root water uptake time-resolved in three dimensions. We present first results for maize plants indicating variations in local root water uptake depending on root type and time of measurement during the day. The results prove the suitability of the method for systematic time-resolved 3D studies of rhizosphere hydraulics including the local water uptake in root systems.

12:15 - 12:30

A MODEL-DATA INTEGRATION STUDY FOR SOIL RICE COLUMN USING MULTISCALE MODELLING APPROACH CONSIDERING RHIZOSPHERE GRADIENTS

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Models of root development and soil resource acquisition often lack representation of nutrient gradients in the rhizosphere. This can be a challenge, in particular for large root systems. In this contribution, we present an application of a newly developed multiscale model to simulate water and phosphate (P) uptake by 3D architectural root systems of upland rice. The model not only simulates the transport at the root system scale

but also captures the dynamics of nutrient gradients around each root segment. To link the model with reality, a large pot experiment was conducted. Rice was grown in large columns using a P deficient soil, having different P concentrations in the topsoil through fertilization and being subjected to contrasting water regimes (permanent field versus drying cycles). At the end of this trial, root architectural parameters and P uptake were determined. In-silico experiments were subsequently set up and carried out under the water regimes and P levels as in the real experiment. The 3D architectural structures of the rice root systems were reconstructed using the phenological root data such as nodal root number, lateral types, interbranch distance, root diameters, root biomass allocation among soil layers. The environmental data such as irrigation and evaporation were used as the boundary conditions for the 3D simulation. The results from the simulations displayed similar trends in total P uptake as the measured ones. The effects of drying cycles and P availability was well represented by the simulated P uptake. More interestingly, the multiscale simulations reveal the underlying processes such as the dynamic of the nutrient depletion zone in the rhizosphere during root growth and nutrient uptake as well as the contributions of specific roots under contrasting scenarios. By model-data, the in-silico experiments enhance the mechanistic understanding of soil-root process across scales, which cannot be discovered by experimental observation only.

12:30 - 14:00 **LUNCH** **(CENTENNIAL HALL)**

14:00 - 14:15 **RHIZOSPHERE 5 & CSSS JOINT SESSION - OPENING REMARKS** **(SALON B+C)**

14:15 - 15:15 **KEYNOTE ADDRESS - AMANDA BLACK** **(SALON B+C)**

AN INDIGENOUS RESPONSE TO A BIOSECURITY THREAT: THE CASE OF KAURI DIEBACK (AGATHIS AUSTRALIS)

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²*BioProtection Research Centre, Canterbury, New Zealand*

In Aotearoa New Zealand the number of biosecurity invasions threatening our culturally important species is increasing. Yet often in the response, the wants and needs of Māori, New Zealand's Indigenous people, are ignored, lost or forgotten in what is a rapidly changing, crowded and confusing biosecurity system. The authority of Māori to respond to biosecurity incursions has been compromised by state-led approaches to governance and management of nature, and is indicative of a lack of integration and respect for Māori cultural practice that has been commonplace throughout the colonial history of New Zealand.

In early 2018 it was announced that Kauri Dieback, caused by the recently named and introduced root pathogen *Phytophthora agathidicida*, had spread rapidly through a significant secondary growth forest, the Waitakere Ranges, close to the urban centre of New Zealand's largest city Auckland. In an attempt to stop the spread of kauri dieback causing disease in their forests, the local tribe *Te Kawarau a Maki* placed a *rāhui* (restriction) on the Waitakere Forest (known to them as Te Wao Nui a Tiriwa). The *rāhui* while supported by

Māori around the world and numerous NGO's, did not receive support from the Auckland Council and therefore no legal support for the *rāhui*.

I will discuss the research to date and significant findings and the role indigenous solutions play in the protection of forests. I will also expand on the role that Te Tira Whakamātaki (the Māori Biosecurity Network) has played in bringing Indigenous Māori communities together to raise awareness of the ongoing cultural impacts that have resulted from biological invasions on top of institutional marginalisation.

15:15 - 15:45 HEALTH BREAK & POSTERS & EXHIBITS (FOYER & SALON A, D, E)

15:45 - 17:45 PARALLEL SESSIONS

SESSION 16: FOREST ECOSYSTEM RHIZOSPHERE (GALLERY A)

Session Chairs: Sue Grayston & Lenka Harantova

15:45 - 16:15

FOREST TREE RHIZOSPHERE: AN ACTIVITY HOTSPOT WITH SPECIFIC MICROBIOME AND SPECIFIC FUNCTIONS

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The tree rhizosphere, the thin layer of soil under the immediate influence of roots, represents the interface between the dominant primary producers of forests and soil, the largest pool of mineral nutrients. Due to the role of the rhizosphere in nutrient exchange, this soil microhabitat is highly specific in terms of chemistry, microbiome composition and function. While the rhizosphere serves as the source of nutrients for roots, it is also rich in organic matter produced by roots as exudates or through root-symbiotic fungi. This root-derived organic matter may represent over 30% of tree primary production.

In temperate and boreal forests with trees forming ectomycorrhizal associations (e.g. *Fagus sylvatica* and *Picea abies*), rhizosphere soils typically have higher microbial biomass and biochemical activity compared to bulk soil. The amount of fungal and bacterial biomass in the rhizosphere is 2-4-fold greater than bulk soil and the same is true for the activity of several hydrolytic and oxidative enzymes. The existence of the rhizosphere is mediated by root activity: while microbial biomass in the rhizosphere remains high during winter when trees are inactive, tree harvest is accompanied by a rapid decrease in rhizosphere microbial biomass and activity.

The specificity of tree rhizospheres with a high proportion of ectomycorrhizal fungi and several bacterial taxa, including Actinobacteria and Proteobacteria, is maintained by the specific chemistry of the rhizosphere composing its metabolome. In terms of function, microbial activity reflects the activity of roots and thus, there is a difference in C utilization and N-cycling processes between rhizosphere and bulk soil, but also between warm seasons and cold winters, when trees are inactive. Although the rhizosphere compartment represents just a small fraction of soil volume, it represents an activity hotspot with specific functions essential for tree nutrition and productivity, as well as for plant-microbe interactions in forest soils.

16:15 - 16:30

COUPLING OF SOIL ZYMOGRAPHY AND AUTORADIOGRAPHY TO QUANTIFY NUTRIENT ACQUISITION IN THE BEECH RHIZOSPHERE

S Spielvogel¹, S Loepmann², T Zilla², J Tegtmeier¹, K Jarosch³, Y Kuzyakov⁴, M Dippold²

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In many ecosystems, plant growth is (co-)limited by phosphorus (P) availability, particularly in forest soils. Soil P can be distributed in very heterogeneous patterns, as the few available studies on that topic suggest. However, no information is available concerning the effects of a homogenous vs. heterogeneous soil P distribution on microbes' and plants' P acquisition strategies in the rhizosphere.

To investigate the interactions among plants, mycorrhiza and microorganisms for P uptake in the rhizosphere we planted beeches in rhizotrones filled with a P-deficient forest soil. The soil was labeled with two different P-sources: Either with $\text{Fe}^{33}\text{PO}_4$, a P source that is rather difficult to mobilize or with isotopically enriched (^{15}N and ^{33}P) microbial necromass. The following P distribution patterns with equal amounts of P applied on each rhizotrone were created: 1) homogenous distribution, 2) 50%-patchiness, and 3) 25%-patchiness to increase the nutrient concentration per area, subsequently resulting in P- hotspots of contrasting intensity.

The microbial P uptake from $\text{Fe}^{33}\text{PO}_4$ was significantly increased with a patchier P distribution. Characteristic PLFAs indicated a massing of ectomycorrhizal fungi associated with beech in $\text{Fe}^{33}\text{PO}_4$ rich patches. These ectomycorrhizal fungi likely increased the P mobilization from P-rich habitats. Habitats with low $\text{Fe}^{33}\text{PO}_4$ content required a more complex microbial community structure in the rhizosphere without a dominant guild to mobilize P. We hypothesize that also a patchy distribution of P-rich microbial necromass will increase plant P-uptake, since roots and mycorrhiza can focus on P mobilizing activity in hotspots. Areas of higher necromass content may cause higher acid phosphatase activity resulting in higher P uptake of plants and microorganisms. The coupling of autoradiography (^{33}P imaging) and zymography addresses the local nutrient uptake in the rhizosphere in time and space as it enables the visualization and the quantification of nutrient fluxes as well as hotspots of microbial activity.

16:30 - 16:45

GREATER ROOT PHOSPHATASE ACTIVITY OF TROPICAL TREES AT LOW PHOSPHORUS SUPPLY DESPITE STRONG VARIATION AMONG CO-OCCURRING SPECIES

X Guilbeault-Mayers¹, E Laliberté¹, BL Turner²

¹*Université de Montréal, Montréal, Québec, Canada*

²*Smithsonian Tropical Research Institute, Ancon, Balboan, Panama*

Soil phosphorus availability in lowland tropical rainforests is a primary determinant of the distribution and growth of tropical tree species. Determining the phosphorus acquisition strategies of tropical tree species could therefore yield insight into patterns of tree beta diversity across edaphic gradients. In particular, the synthesis

of root phosphatases is likely to be of significance given that organic phosphorus represents a large pool of potentially available phosphorus in tropical forest soils. It has also been suggested that a high root phosphatase activity in putative nitrogen-fixing legumes might explain their high abundance in lowland neotropical forests under low phosphorus supply. Here, we measured phosphomonoesterase activity on the first three root orders of co-occurring tropical tree species differing in their nitrogen fixation capacity in soils of contrasting phosphorus availability in lowland tropical forests of Panama. Our results show that root PME activity was higher on average in phosphorus-poor than in phosphorus-rich soils, but that local variation in phosphomonoesterase activity among co-occurring species was larger than that explained by soil phosphorus across sites. Phosphomonoesterase activity was greater for legumes than for non-legumes, but nodulated legumes (i.e., actively fixing nitrogen) did not differ from legumes without nodules. Finally, phosphomonoesterase activity declined with increasing root order, but the magnitude of the decline varied markedly among species, highlighting the importance of classifying fine roots into functional groups prior to measuring root traits. Our results show that phosphomonoesterase activity is greater for trees growing on low phosphorus soils compare with those growing at high phosphorus, although the high local variation in phosphatase activity among co-occurring species points toward a high functional diversity in phosphorus acquisition strategies within individual communities. Our study also supports the idea that the high root phosphatase activity of legumes is a phylogenetically conserved trait with no direct link to nitrogen fixation capacity.

16:45 - 17:00

EFFECTIVE USE OF ORGANIC PHOSPHORUS ALLOWS *XYLOMELUM OCCIDENTALE* TO INHABIT SEVERELY PHOSPHORUS IMPOVERISHED SOILS

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⁴*School of Agriculture, Food and Wine, The University of Adelaide, Urrbrae, South Australia, Australia*

Plants from southwest of Western Australia have adapted to severely nutrient-impooverished soils, in particular, soil phosphorus (P), exhibiting different nutrient-acquisition strategies. Most Proteaceae have the strategy of carboxylate exudation from cluster roots to mine scarcely available soil P. However, not all species in this family have fully mastered this superior P-acquisition strategy. *Xylomelum occidentale* (woody pear) is probably one of them. Unlike its relatives such as *Banksia* and *Hakea* spp., woody pear does not produce functional cluster roots which means that it does not exude carboxylates under low-P condition. It can only be found in some remnant habitats in Southwest Australia. However, no study has investigated why woody pear has a relatively restricted distribution and how it accesses the limited P resource in the soil. Our results show that soil total P pools in woody pear habitats were only marginally higher than those of more common *Banksia* woodlands. The soil organic P accounted for up to three-quarters of soil total P pool in these highly-weathered soils. We then revealed that woody pear depleted at least 65% of organic P in its rhizosphere soil compared with bulk soil. Differences in organic P speciation between woody pear rhizosphere soil and bulk soil were determined using

solution ³¹P nuclear magnetic resonance spectroscopy, to determine which groups of organic P were utilised by woody pear. We surmise that woody pear produces specific phosphatases that are capable of hydrolysing soil organic P effectively, therefore successfully establishing in a niche of a P-impooverished environment that contains slightly more organic P.

17:00 - 17:15

LIVING AND DEAD ROOTS FACILITATE EMERGENCE AND SURVIVAL OF OAK ACORNS IN CENTRAL TEXAS

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Texas is a hotspot for *Quercus* diversity, yet *Quercus* species in Texas are currently in decline due to a multitude of threats including drought, land development, grazing, and oak wilt fungus. On the Edwards plateau in Central Texas, several *Quercus* species form extensive woodlands with *Juniperus ashei*, which together form the critical nesting habitat for rare endemic bird species and play an essential role in the maintenance of key aquifers. Here we established a regeneration experiment to test the impact of living and dead roots on oak acorn emergence and survival in the xeric conditions of the Eastern Edwards Plateau. In November 2013, two hundred *Quercus sinuata* var. *breviloba* acorns were planted in each of three habitat treatments, replicated six times: i) live *Juniperus ashei-Quercus* spp. canopy, ii) dead *Juniperus ashei-Quercus* spp. canopy, iii) open grassland. Both living and dead roots facilitated oak acorn emergence, whereas almost no emergence or survival was observed in the grassland sites. Seedlings under living canopy had a higher number of ectomycorrhizal root tips, significantly shorter roots, higher foliar Ca, Mg, and Mn. The presence of canopy trees, living or dead, significantly enhanced *Quercus* seedling emergence in May 2014. Survival to harvest in October 2017 was significantly associated with increasing *J. ashei* and *Quercus* spp. basal area, shrub cover, and soil organic matter. Seedling biomass and foliar N, P, S were positively associated with dead and open canopy, whereas ectomycorrhizal colonization and foliar Ca, Mg, and Mn were positively associated with live canopy. Thus we infer that the presence of both living and dead roots facilitates *Quercus* emergence, but survival depends on proximity to larger trees, living roots, and canopy. Planting acorns under a *Juniperus ashei-Quercus* spp. canopy, living or dead, is a cheap, sustainable, and effective habitat management practice.

17:15 - 17:30

IDENTIFYING DISEASE SUPPRESSIVE PROPERTIES IN THE RHIZOSPHERE TO PROTECT NEW ZEALAND'S KAURI AGAINST DIEBACK DISEASE

AK Byers¹, A Black¹

¹BioProtection Research Centre, Lincoln, Canterbury, New Zealand

New Zealand kauri (*Agathis australis*) are a coniferous tree species of the ancient family *Araucariaceae*. They are long lived, reaching over 2000 years old, thus making them an integral foundation ecosystem species across Northern New Zealand. At present, only 0.75% of primary kauri forest remains due to heavy deforestation from

forestry and agricultural industries. Now these fragments and trees are under a new threat from an introduced root pathogen *Phytophthora agathidicida* and are now classes as threatened. This pathogen is distributed across the entire kauri range and with no known cure or deterrent, and research is urgently needed for the protection of the remaining kauri forest ecosystems.

Previous research has focused on understanding the pathology of *P. agathidicida*. However, little is understood about the environmental soil factors influencing pathogen virulence and host disease expression. Our aim to identify potential disease suppressive properties in the rhizosphere of old growth forest soils containing asymptomatic trees. The first objective of this project focuses on studying the effects of pathogen invasion on the soil microbial communities of kauri forests, in particular how it varies between diseased and healthy trees. Using high throughput 16s and ITS rRNA sequencing, we have identified differences in the composition of microbial communities from healthy and diseased kauri soils. Additionally, we have compared the microbial communities between kauri and pine soils, to understand how land fragmentation is altering the biotic soil diversity across kauri forests.

Our results show that several fungal and bacterial taxa, such as *Trichoderma*, *Pseudomonas* and *Penicillium* were associated with positive kauri host health. We are now screening these against *P. agathidicida* to assess their biocontrol potential. Additionally, pathogen-seedling bioassays will be conducted and preliminary results discussed regarding how soil microbial communities around the kauri root zone respond to pathogen exposure and seedling disease expression.

17:30 - 17:45

LINKING 31 YEARS OF SPECIES ABUNDANCE DATA FROM A BIODIVERSE MOUNTAIN MEADOW TO PLANT-SOIL FEEDBACK

D in 't Zandt¹, T Herben^{2,3}, A van den Brink¹, E Visser¹, H de Kroon¹

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Plant species co-existence and the subsequent maintenance of high diverse plant communities are long-discussed phenomena in ecology. Currently, interactions between plants and their species-specific soil biota are increasingly understood to play critical roles in these processes. However, attempts to link greenhouse experiments with field data have yielded discrepancies and suggest gaps in our understanding of plant-soil biota interactions as a possible key component in species co-existence processes.

The interaction between plants and soil biota is generally studied within the concept of plant-soil feedback: soil property changes resulting from plant growth, which affect future plant growth on that same soil. We combined experimental plant-soil feedback data with unique field data from a species-rich mountain meadow containing species abundance measurements over 31 consecutive years for 24 co-existing plant species. We show a time dependency in the relation between plant-soil feedback and plant relative abundance in the field. In some years, species with negative plant-soil feedback in the experimental setting were in high abundance and positive plant-soil feedback species in low abundance in the field, whereas in other years no relation occurred.

Moreover, we observed a semi-cyclic relation between species increase and decrease in relative abundance and its plant-soil feedback. This complies with current theoretical frameworks suggesting that negative plant-soil feedback averts monodominance of successful plant species in a cyclic fashion. Such self-limitation of successful species likely creates opportunities for species with lower competitive ability to persist in the same community, thus maintaining diverse plant communities. Our results thus suggest that plant-soil feedback plays an essential, consistent role in plant species co-existence processes.

SESSION 17: ROOT IMAGING AND PHENOTYPING

(GALLERY B)

Session Chairs: Christopher Topp & Randy Clark

15:45 - 16:15

3D IMAGING, COMPUTER VISION, STATISTICAL AND MATHEMATICAL APPROACHES REVEAL THE GENETIC BASIS OF PLANT ROOT AND INFLORESCENCE ARCHITECTURES

CN Topp¹

¹Donald Danforth Plant Science Center, St. Louis, United States

The Topp Lab is interested in crop root system growth dynamics and function in response to environmental stresses such as drought, rhizosphere competition, and as a consequence of artificial selection for agronomically important traits including nitrogen uptake and high plant density. Studying roots requires the development of imaging technologies, computational infrastructure, and statistical methods that can capture and analyze morphologically complex networks over time and at high-throughput. The lab uses several imaging tools (optical, X-ray CT, PET, etc.) along with quantitative genetics and molecular biology to understand the dynamics of root growth and whole plant physiology. We aim to understand the relationships among root traits that can be effectively measured both in controlled laboratory environments and in the field, and to identify genes and gene networks that control root, and ultimately whole plant, traits useful for crop improvement. I will discuss our current capabilities and several examples of how cutting edge phenomics approaches are rapidly improving our understanding of the genetic basis of root and inflorescence architecture in maize, sorghum, *Setaria*, and grapevine.

16:15 - 16:45

APPLICATION OF ROOT IMAGING AND PHENOTYPING TO MODELING AND BREEDING

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²University of Queensland, Brisbane, Queensland, Australia

Root imaging and phenotyping can bring enhanced knowledge to breeding processes. These tools and techniques can be incorporated and leveraged through many ways. This talk will focus on examples of how root phenotyping can be applied directly as well as indirectly through the combination of phenotyping tools with modeling to help guide breeding efforts and bring additional insight into GxExM interactions.

16:45 - 17:00

FUNCTIONAL SIGNIFICANCE OF HETERORRHIZY IN A ROOT SYSTEM FOR WATER UPTAKE IN RICE PLANT

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A root system of rice plant consists of mainly three component roots including main roots (one seminal root and nodal roots), L-type and S-type lateral roots, which have different morphological and anatomical features. Such phenomenon is termed as heterorhizy. For instance, main roots have larger diameter as compared with the two types of laterals, and branch L-type and S-type lateral roots. L-type lateral root is thicker and longer than S-type lateral root, and moreover, has an ability to branch higher order lateral roots whereas S-type lateral roots do not have such ability. They are different also in dimension of vascular system. It is therefore assumed that each component root has different impact on water uptake. In this aspect, this study aimed to examine the contribution of each component root to water uptake of the whole root system by measuring hydraulic conductance and conductivity of the root system as well as the surface area of each component root. Results showed an upland variety, IRAT 109, which was grown till two weeks after heading, had higher ratio of surface area of S-type lateral root in the whole root system and higher root hydraulic conductance and conductivity under different water stress conditions than other varieties studied. Furthermore, it was observed that S-type lateral root showed intensified lignin and suberin deposition on the cell wall, which may increase hydraulic resistance, while it forms no aerenchyma and has less cell layers in radial directions than the other component roots, which are assumed to result in decreased hydraulic resistance. These results indicate that the contribution of each component root may be different from each other, and S-type lateral root may have higher hydraulic conductivity than main root and L-type lateral root, and thus may contribute most to an increased hydraulic conductance of a root system.

17:00 - 17:15

THE PLASTICITY OF CANOLA ROOT SYSTEM ARCHITECTURE: HYDROPONICS VERSUS SOIL GROWN CANOLA

HP Ahmed², J. Mora-Macias¹, D. Schneider¹, L. Kochian¹

¹ *Global Institute for Food Security (GIFS), UofS, Saskatoon, SK, Canada*

Canola (*Brassica napus*) is one of the most important oilseeds worldwide and Canada as it is an excellent source of health-promoting oils and high-quality protein. Hence, there is considerable research focused on improving canola productivity and resiliency. This is a real need to improve canola root system architecture (RSA), because it has not been a focus on plant breeders, due to difficulties in seeing root traits in the field. We are finding that the canola RSA is amazingly plastic and can change dramatically in response to the structure, chemistry, microbiology and mineral availability of the root growth medium. We grew two canola varieties (VT500G and DH12075) in hydroponic and gel-based growth platforms with 2D and 3D root imaging platforms. In hydroponics, canola produced an extensive root system of fine primary and lateral roots. This is similar to what we see for other dicot species grown in hydroponics (soybean, cucumber, Arabidopsis). These other dicot species produce similar fine root system architectures (RSA) when grown in soil compared to hydroponics.

However, soil-grown canola produced root systems dominated by thick primary root with thick lateral roots and a few fine roots. We are investigating the agronomic and molecular developmental nature of this plasticity, as we see this as an opportunity to possibly improve the nutrient and water acquisition properties of soil grown roots via production of more fine roots in the system. We are looking at the role of soil fertility/nutrition, mechanical impedance of the growth medium, and the role of the root microbiome in this root plasticity. One thing we have found in hydroponics is that improving the iron (Fe) nutrition of the hydroponic media does lead to somewhat thicker primary and lateral roots, so they begin to look more like soil grown roots. The findings from these studies will be presented.

17:15 - 17:30

HIGH-THROUGHPUT PHENOTYPING OF MULTIPLE ION UPTAKE KINETICS IN MAIZE

M Griffiths¹, H Guo¹, A Seetheapalli¹, LM York¹

¹Noble Research Institute LLC, Ardmore, United States

Zones of nutrient depletion are fundamental aspects of the rhizosphere, yet little is known about genetic variation among plants for root nutrient uptake ability. The aim of this study is to close the knowledge gap between root and function with the development of a new modular platform for high-throughput phenotyping of multiple ion uptake kinetics (MIUK), which aggregates phenes (units of phenotype) relating to ion transporter affinity, transporter number and other processes. After growing in a common hydroponics chamber, seedlings are transferred to individual measurement vessels that have an inlet for stock nutrient solution, and an outlet for sampling over time that are both controlled by 24-channel peristaltic pumps. Using ion chromatography, concentrations for multiple anions and cations are determined for each sample which allows calculation of net influx. This allows characterizing uptake rates for multiple macronutrients that can limit crop productivity including nitrate, phosphate and potassium. Using this system, uptake rates for multiple ions have been characterized for 25 parental lines from the maize nested association mapping (NAM) population and the heritability determined. The parents with maximum phenotypic distance will inform selection of a recombinant inbred line population for identifying novel genetic associations with MIUK. In turn, this work will further our understanding of MIUK, parameterize models such as OpenSimRoot to understand how depletion zones influence the rhizosphere, and identify targets for facilitating breeding efforts for improved nutrient acquisition.

Session Chair: Steve Siciliano

15:45 - 16:00

A ROOT TO SUCCESS: HARNESSING THE NATURAL COMPLEXITY OF RHIZOSPHERE EXUDATION TO DECONTAMINATE SOIL

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Pollution of soils with heavy metals is a worldwide problem generating substantial risks to human health and the environment. Sustainable phytoremediation strategies are trying to harness natural complexity which allows certain plants to decontaminate land. One essential mechanism used by plants is thought to be exudation of metabolites aimed at modifying challenging soil environments. However, the extent and variation of root exudation remains largely obscure in many important crops.

To explore the impact of metabolite exudation, a novel small-scale but highly reproducible growth system was developed at the Institut de Recherche en Biologie Végétale de l'Université de Montréal, alongside larger-scale pot trials which are readily trackable for study of a diverse range of heavy metal contaminants within sand, constructed and real-world soils. Four plant species representing different agronomically important functional traits are being extensively scrutinised to identify the predominant classes of exuded compounds, and ultimately the different strategies taken in response to contamination.

These species comprise a metal hyperaccumulating grass (*Festuca arundinacea*), nitrogen-fixing legumes (*Medicago sativa*, *Lupinus albus*) and a fast growing woody crop (*Salix miyabeana*). Exudation patterns revealed that phenolic, carbohydrate and organic acid profiles vary substantially. Of all four species, *L. albus* exudes the largest amounts in the rhizosphere, suggesting extensive capabilities to influence their soil environment.

The comparison of distinct species subjected to different forms and concentration of contaminants allows important insight into how root exudation affects the heavy metal bioavailability and transport inside the plants. Observation of different potential exudation strategies will bring new knowledge on how investment of resources in the rhizosphere can help plants tolerate, or even overcome, the effect of anthropogenic pollution upon the natural environment.

Understanding these natural solutions is vital in helping to devise sustainable land management strategies to reduce the long term impact of human industry on soils around the world.

16:00 - 16:15

RHIZOREMEDIATION OF TOLUENE USING HYBRID POPLARS AT A PILOT INDUSTRIAL FIELD SITE

M Ben-Israel¹, K Khosla¹, JZ Habtewold^{1,2}, DT Tsao³, KE Dunfield¹

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²*Agriculture and Agri-Food Canada, Ottawa, ON, Canada*

³*BP Corporation North America, Inc., Naperville, IL, United States*

Rhizoremediation and phytoextraction of toluene were assessed in an urban pilot system, where hybrid poplars were planted in 2008 to remediate legacy impacts to an underlying shallow fractured bedrock aquifer. Microbial degrader populations were studied *in situ* through excavation of root and rhizosphere samples. Toluene degradation genes (PHE, RMO, TOD, and *bssA*) detected in soil using quantitative PCR (10^4 – 10^6 copies g^{-1} dry soil) confirmed potential toluene biodegradation in the rhizosphere. Meanwhile, root-associated microbes had relatively larger degrader populations (10^5 – 10^6 TOD gene copies g^{-1} dry root) in trees overlying the toluene source zone. Sequencing of root 16S *rRNA* genes showed that the *Streptomyces* genus (which contains degrader species) dominated in these same trees. Moreover, bioinformatic predictions of functional content using PICRUSt software showed that 16S *rRNA* sequences associated with toluene degradation capacity (2.78×10^5 – 3.68×10^5 reads) were also maximal overlying the source zone. Together, these microbial results were consistent with a parallel study of toluene phytoextraction (uptake and translocation) by the stand: trees overlying the source zone had lower detectable *in planta* toluene concentrations than would be expected without the influence of rhizoremediation. Finally, we incubated rhizosphere soil with ^{13}C -labeled toluene in a DNA stable isotope probing microcosm study and used isopycnic separation of extracted DNA to generate fractions over a range of densities. Denaturing gradient gel electrophoresis analysis targeting bacterial 16S *rRNA* and fungal 18S *rRNA* genes revealed unique community profiles for both targets in fractions predicted to contain ^{13}C -labeled DNA. Next, we will sequence these amplicons to taxonomically identify microbial toluene degraders. Overall, we have demonstrated active rhizoremediation and phytoextraction occurring at this site and are working to expand the breadth of *in situ* metagenomic assessment approaches for future applications.

16:15 - 16:30

COMPLEXITY MATTERS: SOIL FOOD WEBS SHAPE THE ROOT MICROBIOME AND MODIFY PLANT TRAITS IN WILLOW UNDER CONTAMINATED CONDITIONS.

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The degradation of organic contaminants around plant roots, rhizodegradation, principally depends on the plant-root microbiome (holobiont) interaction. There is mounting evidence suggesting that the diversity of the contaminated soils is linked to efficient phytoremediation. Indeed, most of our understanding regarding the degradation process comes from *in vitro* and controlled greenhouse experiments. These experiments often ignore the influence of other soil community members. However, a significant portion of the soil biomass is

composed of soil invertebrates that dwell in the root environment. Thus, soil invertebrates may alter the effectivity of rhizodegradation by modifying the synergy between roots and microbes. Therefore, the aim of this project was to determine whether the complexity of the eukaryotic soil food web is a significant factor influencing the productivity of the willow through the alteration of the microbial community under phenanthrene contamination. We hypothesized that willows inoculated with a more complex invertebrate food web will thrive better than willows that rely only on microbial communities or less complex food webs. A 6L pots full factorial experiment with 2 factors/6 replicates was carried out. The contamination factor had two levels: 100mg·kg⁻¹ phenanthrene and 0mg·kg⁻¹. The diversity factor had eight levels: microbial community (B), nematodes (N), springtails (C), earthworms (E), N~C, N~E, C~E, C~E~N. After 6 weeks of growth, plant shoots, roots and rhizosphere and bulk soil were sampled for amplicon sequencing analysis, plant morphological traits and phenanthrene levels. Our results show significant differences in biomass production for diversity treatments and will highlight relative abundance changes in microbial taxa when facing contaminant stress and diversity treatments. This experiment will also inform us which diversity treatments are more efficient in promoting phenanthrene degradation.

16:30 - 16:45

RHIZOREMEDIATION OF ORGANIC CHEMICALS IN THE EXISTENCE OF FE USING EXUDED H₂O₂ BY FENTON REACTION

T Wagatsuma¹, S Tomita¹, T Ezura¹, A Kikuchi¹, S Namiki¹, T Otani², S Seike², K Tawaraya¹

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We previously found that bur marigold (hygrophyte, Asteraceae, *Bidens tripartita* L.) had considerably high concentration of H₂O₂ in the root apoplast and could decompose the rhizosphere CH₄ immediately in the presence of Fe. Methane was decomposed with hydroxy radical (·OH) or ferryl ion (Fe(IV)=O²⁺) synthesized by the Fenton's process using root apoplastic H₂O₂ and the medium Fe. Here, we report the significantly high rhizosphere-remediating potential of herbicide atrazine (ATZ) and other chemicals especially in Asteraceae and Pedaliaceae plants by the application of 0.2 mM Fe. Root apoplastic H₂O₂ was detected moderately high in all plant species tested in Lamiaceae and considerably high in Asteraceae and Pedaliaceae, however, only slight in Valerianaceae, Malvaceae and Basellaceae, and no detection in Poaceae, Cucurbitaceae, Brassicaceae, Fabaceae and other 10 families. Qualitative analyses using inhibitors suggested the contribution of apoplastic oxidative cycle connected with NADPH oxidase and SOD and aquaporin-type H₂O₂ transporter to the greater apoplastic H₂O₂. *In vitro* system, ATZ and other organic chemicals (herbicide Basagran, DNP, catechol, methylene blue, SDS) were exponentially and immediately decreased depending either on the concentration of 0 – 0.2 mM Fe(II) or 0 – 1 mM H₂O₂ in the medium at pH 5, and remarkable decrease in ATZ was observed at pH 6.5 mimicking upland field conditions irrespective of the Fe(III) forms (picolate, NTA, humate, benzoate, EDTA, EDDHA, pyrophosphate, phenylacetate, rhodizonate, citrate). *In vivo* system, significantly greater decrease in ATZ was observed immediately in the presence of Fe(II) or Fe(III) in Asteraceae and Pedaliaceae plants. The less toxic degradation product, propazine, was detected in the medium after the treatment. This is

the first report on the decomposition of the organic chemicals by the plant roots based on the Fenton reaction using Fe(II) or Fe(III) in the rhizosphere. We newly termed this reaction as Rhizo-Fenton reaction.

16:45 - 17:00

EXTRACELLULAR SILICA NANO-COAT INDUCED BY POLYETHYLENEIMINE CONFERS ALUMINUM TOLERANCE TO ROOT BORDER CELLS

XY Chen¹, XW Li¹, YM He¹, F Baluška², JB Pu³, LJ Wang³, V Demidchik¹, S Shabala¹, **M Yu**¹

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Silicon (Si) plays a number of roles in various organisms. One of the obvious examples is the use of biomineralized silica as an outer shell for protection from environmental stresses. According to diatom biosilicification, polyethyleneimine (PEI), a water-soluble polyamine, can induce the formation an extracellular nano-coat of silica on the surface of plant cells. However, it is unclear whether the artificially designed nano-coat can also improve plant tolerance to toxic metals. In this study, we have addressed this question by using PEI-induced nano-silica (PEI-Si) to coat root border cells (RBCs) of *Pisum sativum*, to enhance Al tolerance. We found that formation of PEI-Si on the surface of RBCs increased the viability of roots cells treated by toxic Al³⁺. Atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), Laser-Scanning Confocal Microscopy (LSCM) were applied to investigate the mechanism of silica nano-coat-induced tolerance to Al³⁺ in RBCs. Tests with JC-1 and CM-H₂DCFDA showed that, in the presence of Al³⁺, the formation of extracellular PEI-Si contributed to maintaining the integrity of the mitochondrial membrane and reduced the production of reactive oxygen species (ROS). The nano-coating also increased the Al³⁺ absorption capacity of cells. Both AFM and XPS results demonstrated that the formation of PEI-Si increased the strength of the cell wall, and the capability of the Al³⁺ accumulation on cell surfaces. Our results provided evidence that PEI-Si induces the tolerance of RBCs to Al³⁺ by binding Al³⁺ in the silica-coat, thus reducing the entry of Al³⁺ into the cytoplasm.

17:00 - 17:15

SOIL FUNGI, A RESOURCE AGAINST CADMIUM THREAT IN CACAO PLANTS

J Caceres¹, **H Cordoba**¹, E Torres¹

¹*Universidad Nacional de Colombia, Bogotá, Colombia*

Cadmium is a heavy metal present in soils due to geological and anthropogenic causes. Some cultivated plants accumulate cadmium into edible parts causing health problems. Despite American cacao is recognized by its quality and aroma, the presence of cadmium has been reported in some chocolate products. An agronomic strategy is using soil fungi to mitigate the absorption of cadmium by the plant. However, knowledge about cadmium-resistant fungi present in cacao rhizosphere is scarce. This study aimed to make a morphological and molecular identification of cultivable cadmium-resistant fungi; characterize their growth, functionality, tolerance index, minimum inhibitory concentration, and cadmium accumulation. Soil samples from cacao rhizosphere were collected in locations with different levels of cadmium in Colombia. Fungi were isolated in

solid media with 6 mg kg⁻¹ cadmium and a diversity analysis was made. Their ability to degrade cellulose and to solubilize phosphates was assessed with differential culture media. Resistant strains to 50 mg kg⁻¹ cadmium were used for further evaluations. The location with the highest available level cadmium (16.5 mg kg⁻¹) showed a higher diversity. The 93.3% of isolated fungi belong to Ascomycota division and many morphotypes had cellulose degradation and phosphate solubilization simultaneously. Cadmium reduced *Metarhizium* sp., *Trichoderma* sp., and *Rousoella siamensis* growth. Furthermore, cadmium induce *Massarina igniaria* mass accumulation, showing the highest cadmium accumulation (10.17 mg g⁻¹) with a tolerance index about 0.8 and a minimum inhibitory concentration of 1000 mg kg⁻¹. *Metarhizium* sp. had a minimum inhibitory concentration of 6000 mg kg⁻¹. Overall, the results contribute to the knowledge about cadmium-resistant fungi related to cacao rhizosphere. The characterization of these promising strains is a key step to study mechanisms of resistance and develop biotechnology-based strategies.

SESSION 19: ROOT-SOIL INTERACTIONS

(GALLERY D)

Session Chairs: Bobbi Helgason & Steve Mamet

15:45 - 16:00

DOES P CYCLING CHANGE OVER THE GROWING SEASON IN WHEAT RHIZOSPHERE AND BULK SOILS IN LONG-TERM PLOTS WITH DIFFERENT N AND P FERTILIZATION?

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Biological phosphorus (P) cycling processes, such as enzyme activities, should differ between rhizosphere and bulk soil. For agricultural crops these differences may vary depending on the plant growth stage. In 2017, rhizosphere and bulk soils were collected bi-weekly from May to September from long-term plots in Swift Current, SK. The six treatments were: continuous wheat established in 1967 with N and P fertilization (CW+N+P) or with P fertilization alone (CW-N+P); a lentil-wheat rotation established in 1982 with N and P fertilization in the wheat phase only (WL+N+P); and sub-plots for these plots, where P fertilization stopped in 1995 (CW+N-P, CW-N-P) or 2008 (WL+N-P). Samples were analyzed for total P, total organic P, anion-exchange resin P (resin-P), bicarbonate- and CaCl₂-extractable P, P enzyme activity (acid and alkaline phosphomonoesterase, phosphodiesterase) and pH (CaCl₂). There were no significant treatment*date*soil position (rhizosphere v. bulk) interactions. Treatment significantly influenced many aspects of P cycling, with significantly lower pH in soils with long-term N fertilization, and lower P concentrations where P fertilization was stopped. Rhizosphere soils were significantly different from bulk soils for bicarbonate-P, acid phosphomonoesterase and phosphodiesterase, with some date*soil position interactions, indicating that plant growth stage will influence rhizosphere studies of crop plants.

16:00 - 16:15

LONG-TERM CROP ROTATION DIVERSITY AFFECTS FUNCTION AND STRUCTURE OF THE SOIL AND RHIZOSPHERE MICROBIAL COMMUNITIES

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Crop rotation diversity is important for agroecosystem health. In addition to diversifying economic risk and breaking disease cycles, they provide variety in plant resource demands and quality of residue returns. We surveyed two long-term field experiments comparing continuous cereals with diverse crop rotations in a humid corn-based system and a semi-arid wheat-based system. Edaphic factors including soil organic matter (SOM) composition, extracellular enzyme activities (EEA), and nutrient availability differed in continuous vs. rotation cereal production. Amplicon-based 16S rRNA and ITS gene profiling of root, rhizosphere, and bulk soils likewise showed significant differences in microbial community structure. Although no differences were detected in simple alpha diversity, certain taxa in the bulk and rhizosphere soils increased in abundance (e.g. Actinobacteria; Sordariomycetes) particularly under continuous cereals. Differences were greater in the corn vs. wheat system and were correlated with edaphic factors such as microbial EEA (phosphatase and β -glucosidase) and available nutrient concentrations. Mineralizable C was lower and lignin accumulated in continuous wheat and corn indicating that there was greater turnover of recent plant C inputs in diverse rotations. After 20 years, crop rotation selected for distinct bulk and rhizosphere communities, and affected key soil properties, including microbial nutrient turnover.

16:15 - 16:30

DOES CROP FREQUENCY AND DIVERSIFICATION IN CANOLA-PULSE-CEREAL ROTATIONS CHANGE THE ARBUSCULAR MYCORRHIZAL FUNGAL MICROBIOME IN CROP ROOTS AND RHIZOSPHERE AND CROP YIELD?

J Masse^{1,2}, C Hamel³, L Bainard⁴, Y Gan⁴, E Yergeau⁵, C Greer⁶, M Hijri^{1,2}, M St-Arnaud^{1,2}

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With \$26.7 billion injected into the economy each year, canola is the most profitable crop in Canada. In western Canada, canola is grown in rotation with other crops, being most often cereals and pulses. However, the widespread use of canola, a non-mycotrophic plant, in rotations raises concerns on the loss of soil microbial diversity and increased susceptibility of crops to pathogens. The effect of the frequency of each rotation-species on the microbiome and productivity of each crop are still poorly understood. The main objective of this research

is to characterize the AMF community of wheat, pea and canola under different rotation systems in order to understand the effects of crop frequencies on the root and rhizospheric microbiome of each crop and on plant productivity. To do this, three rotation systems (intensifying canola, cereals or pulses over four years) were tested in a complete random blocks design. The root and rhizosphere microbiomes were sampled for each of the rotation phases at two growing stages. The rotation system and the growth stages had an impact on the biodiversity and structure of AMF communities in all crops. On the other hand, only wheat has seen its productivity increased in the pulse-intensified rotation.

16:30 - 16:45

DISCOVERY OF THE UNTAPPED POTENTIAL OF H₂-OXIDIZING BACTERIA IN SOIL THROUGH THE USE OF MULTI-OMICS, MICROBIOLOGICAL AND MODELLING APPROACHES

P Constant¹, M Khdiri¹, S Piché-Choquette¹, J Saavedra-Lavoie¹, A de la Porte¹

¹*INRS, Centre Armand-Frappier Santé Biotechnologie, Laval, Québec, Canada*

Local accumulations of molecular hydrogen (H₂) can be found in soil, especially in the rhizosphere of legume plants, where H₂ is an obligate by-product of nitrogen fixation. The enrichment of H₂-oxidizing bacteria (HOB) in these microenvironments has been shown to have a fertilization effect on several different crops. The benefit of HOB is attributed to their production of plant growth-promoting factors, yet their interactions with other members of soil microbial communities have received little attention. Here we report that the energy potential of H₂, when supplied to soil, alters ecological niche partitioning of bacteria and fungi, with multifaceted consequences for both generalist and specialist microbial functions. Dynamic microcosms were used to expose soil to environmentally-relevant H₂ mixing ratios ranging from typical atmospheric level (0.5 ppmv) to soil-nodule interface (10,000 ppmv). In addition to stimulate HOB, H₂ enhanced community level carbon substrate utilization potential and lowered both CO and CH₄ uptake activities in soil. A theoretical framework modelling H₂ decay as a function of distance from H₂-emitting point sources suggest that H₂ impact on soil on biogeochemical processes extends the boundaries of the rhizosphere. We postulate that metabolic flexibility and biological interactions of HOB are drivers of H₂ fertilization effect.

16:45 - 17:00

EVALUATION OF SOIL BACTERIA AS BIOCONTROL AGENTS FOR FIELD PEA ROOT ROT CAUSED BY APHANOMYCES EUTEICHES

AT Godebo¹, FL Walley¹, JJ Germida¹

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The soilborne disease aphanomyces root rot, for which management is a challenge because of insufficient and ineffective control options, continue to impose significant threats to western Canadian farmers both in productivity and quality of the field pea crop. Therefore, the aim of this study was to isolate biocontrol bacteria from pea field soils across Saskatchewan and investigate the potential to suppress aphanomyces root rot in field pea in growth chamber conditions. The study comprised in vitro laboratory assays and in vivo growth chamber experiments. The zone of mycelia growth inhibition and percent of zoospore germination inhibition

were considered as a measure of antagonistic potential. Initial in vitro screening of soil bacteria identified 184 isolates (22 from an existing culture collection) that inhibited mycelia and zoospore developmental stages of *A. euteiches*. The mean mycelial growth inhibition potential ranged from 1 mm to 12 mm whereas the mean zoospore germination inhibition ranged from 0 to 100 percent. Based on 16S rDNA gene sequencing, the antagonistic bacterial isolates were placed into 18 different genera. In vivo growth chamber-based screening of 47 potential biocontrol bacteria identified isolates such as K-Hf-L9 (*Pseudomonas fluorescens*), PSV1-7 (*Pantoea agglomerans*) and K-Hf-H2 (*Lysobacter capsici*) as having the highest ($\alpha = 0.05$) biocontrol efficacy against aphanomyces root rot in field pea. Based on the findings, this study has now transitioned to evaluate biocontrol efficacy under field conditions across a range of Saskatchewan soil types. Moreover, investigation of the mechanism by which antagonism/biocontrol was achieved and biocontrol efficacy towards other root rot complex pathogens of field pea and other susceptible crops are part of the future study considerations.

17:00 - 17:15

POTENTIAL OF CHERRY RHIZOSPHERE ACTINOMYCETES AS BIOCONTROL AGENTS AGAINST PLANT-PARASITIC NEMATODES

M Marin-Bruzos¹, T Voegel², S Grayston¹, M Edgley³, D Moerman³, T Forge⁴, L Nelson²

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Replant Disease is a major problem affecting the establishment of new cherry orchards in the Okanagan valley, B.C., Canada. The term is used to describe the commonly observed poor growth and delayed cropping of young cherry trees planted in old apple orchard soils. Replant disease is caused by a mixture of pathogenic organisms (nematodes and fungi) and other soil factors, such as pH and texture. In this research, the potential of microorganisms isolated from cherry rhizospheres as plant growth promoters or biocontrol agents was evaluated. Seventy-six bacterial isolates were purified from cherry rhizospheres. The primary screening for nematode biocontrol activity performed on *Caenorhabditis elegans*, showed 13 strains of actinomycetes that paralyzed or repelled the nematodes. These isolates were screened for plant-parasitic nematode (*Pratylenchus penetrans*) control activity on onion seedlings. The results revealed eight strains that reduced nematode infection on onion roots by more than a 50% compared to the non-inoculated control plants. Plant-growth-promoting traits of the isolates, for example, the production of lytic enzymes, biofilm formation and antifungal activity were studied. This research demonstrates that rhizobacteria from the cherry rhizosphere have potential to be employed as microbial inoculants for biocontrol of replant disease.

17:15 - 17:30

DIVERSITY OF PARASITIC FUNGI FROM SOYBEAN CYST NEMATODE ASSOCIATED WITH LONG-TERM CONTINUOUS CROPPING OF SOYBEAN IN BLACK SOIL

Y Xu¹, J Song¹, S Li¹, W Wei¹, Q Yao¹, F Pan¹

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²Crop Genetics Research Unit, United States Department of Agriculture-Agricultural Research Service, Stoneville, MS, United States

The soybean cyst nematode (SCN) is a major yield-limiting pest of soybean. In this study, experiments were conducted to examine the diversity of parasitic fungi from SCN associated with disease-suppressive soil fields in Northeast China. Soil samples were collected from three fields under different rotation systems that were established in 1991: (1) a continuous long-term cropping field with soybean (SSSS) that had been shown to be SCN-suppressive, (2) cycles of three-year rotation with corn, soybean, and wheat (WCS), and (3) continuous cropping field with three-year cycles of two years soybean and one-year corn (SSC). In the traditional method result, cyst densities of SCN declined as increase of parasitic fungi, and the percentage of parasitic fungi associated with cyst of SCN was higher in SSSS field than other two fields. PCR-DGGE also showed that parasitic fungi of SCN were also increased in SSSS field, compared with the other two fields. Principal component analysis based on PCR-DGGE data revealed that fungal communities on cysts could be divided into three groups: one group occurred in SSSS, and the other two groups were in WCS and SSC fields, respectively. Long-term cropping with soybean monoculture in the black soil field might increase parasitic fungi of SCN. These fungal communities may play an important role in the ecological suppression of SCN in disease-suppressive soil.

18:00 - 21:00

JOINT RECEPTION WITH CSSS

(REMAI MODERN ART GALLERY)

Thursday, July 11th, 2019

08:30 - 09:30

KEYNOTE ADDRESS - MICHELLE WATT

(SALON B+C)

RHIZOSPHERE DYNAMICS AND INTERVENTIONS TO INCREASE CROP PRODUCTIVITY

M Watt^{1, 2}

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Selection of beneficial rhizospheres is an opportunity to make fast gains in productivity because plant genetics, abiotic and biotic soil conditions, and management are expressed together in one phenotype. Our aim is to discover, pre-design and generate beneficial rhizospheres in fields and to manage them in real time during the sown crop. To do this, we use two conceptual frameworks: rhizosphere phenotypes (that include the root), and rhizosphere dynamics. Rhizosphere phenotypes that benefit crop productivity (yield per resource) have arisen on farms in the past from post hoc interactions among plant genotype, soil management, root-zone interventions, and climate. Root, soil, chemical and microbiology disciplines were siloed in the latter half of the 20th century mainly due to reductionist approaches. We are working across those disciplines. Today we use non-destructive, multi-sensor imaging technologies, models, and new experimental approaches, to put reductionist knowledge into rhizosphere systems, including roots and shoots. This talk will cover examples of the projects we are undertaking towards beneficial rhizosphere phenotypes, and the dynamic framework we use to address spatial and temporal heterogeneity to meet the challenge of translating rhizosphere science to resource-efficient farming systems.

09:30 - 10:00

HEALTH BREAK & POSTERS

(FOYER & SALON A, D, E)

10:00 - 12:00

PARALLEL SESSIONS

SESSION 20: MUTUALISMS AND SYMBIONTS

(GALLERY A)

Session Chairs: Louise Nelson & Miranda Hart

10:00 - 10:30

THE FATE OF FUNGAL BIOFERTILIZERS – WHERE DO THEY GO, AND HOW DO THEY GET THERE?

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The use of fungal biofertilizers is common place, yet there is little data surrounding the spread of biofertilizers into the natural environment. Commercial inoculants may pose a threat to local fungal communities, which may not be resistant to alien introductions. Unfortunately, long term data on the fate and effects of commercial fungal inoculant use are lacking.

We evaluated the establishment and spread of a commercial AMF inoculant in a variety of agricultural and natural settings to evaluate its value as a biofertilizer and its threat to indigenous fungal communities, including: vineyards, grain cropping systems and natural grasslands. We also looked at corresponding changes in plant performance and community.

Our results show that while inoculant establishment occurs in some cases, it is not easily predictable. Inoculant spread beyond point of introduction was also difficult to predict and was influenced by both Indigenous soil and plant communities. In all cases, there was inconsistent evidence for crop benefit.

Given the ubiquity of commercial inoculant use, and its ability to establish and spread from point of introduction, the use of fungal inoculants should be carefully evaluated.

10:30 - 10:45

ESTABLISHMENT AND FUNCTIONALITY OF ARBUSCULAR MYCORRHIZAL COMMUNITIES IN THE ROOT RHIZOSPHERE

H Bücking¹, K Garcia², K Cope¹, A Kafle²

¹*South Dakota State University, Brookings, United States*

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Arbuscular mycorrhizal (AM) fungi are among the most critical soil organisms in the rhizosphere and form symbiotic associations with the majority of land plants. These beneficial soil fungi increase the uptake of nutrients, such as phosphate, nitrogen, and potassium, and improve the resistance of plants against many abiotic (e.g. drought, salinity, heavy metals) and biotic (root pathogens) stresses. In return for their beneficial effect on nutrient uptake, a host plant allocates up to 20% of its photosynthetically fixed carbon to these root symbionts. The extensive extraradical mycelium of the AM fungus and its mycorrhizosphere serves as conduit for plant derived carbon resources and provides an important ecological niche for diverse microbial communities in the soil, and leads to quantitative and qualitative changes in the microbial community composition. Associations of AM fungi with their host plants are many-to-many interactions; each host plant is colonized on a small spatial scale with diverse communities of AM fungi, and each fungus can colonize multiple host plants and connect plants via common mycorrhizal networks. However, AM fungi differ greatly in the benefit that they provide for their host plant, but it is currently unknown how AM fungal communities are composed and what mechanisms control the AM community composition of plants. We will present results of our recent experiments that clearly demonstrate the role that the plant nutrient demand plays in the AM fungal community composition, and how AM fungi that are less beneficial for the host can gain a competitive advantage in AM fungal communities over fungi that are highly beneficial for the host. We will correlate these findings to changes in the plant's gene expression in response to the colonization with low or high benefit AM fungi and the processes that could allow host plants to control their AM community composition in the rhizosphere.

10:45 - 11:00

MICROBIAL COMMUNITY COMPOSITION AND SUCCESSION IN THE HYPHOSPHERE OF AN ARBUSCULAR MYCORRHIZAL FUNGUS

BD Emmett¹, V Levesque-Tremblay¹, MJ Harrison¹

¹*Boyce Thompson Institute, Ithaca, United States*

Arbuscular mycorrhizal fungi are obligate plant symbionts that associate with approximately 80% of all land plants. Fungal extraradical hyphae forage for nutrients and extend the influence of root-derived carbon into the soil environment, driving changes in microbial community composition and rates of nutrient cycling in the hyphosphere. Attachment of particular bacterial strains to the hyphae has been demonstrated *in vitro*, but the composition and function of microbial communities closely associated with extraradical hyphae in soil remains largely unexplored.

Experiments were undertaken to detail the composition of bacterial communities closely associated with mycorrhizal fungal hyphae in three distinct agricultural soils and to investigate the temporal dynamics of community establishment and succession following hyphal colonization of the soil. Extraradical hyphae of *Glomus versiforme* in symbiosis with *Brachypodium distachyon* were allowed to colonize an in-growth core (25 mm mesh) containing a sand and soil mixture (3:1 w/w). Following colonization, the sand/soil matrix was submerged in sterile water and hyphae collected under a dissecting scope. DNA from hyphal samples and bulk soil controls were extracted and 16S rRNA gene amplicon libraries sequenced on the Illumina MiSeq system. Hyphal attached communities diverged from bulk soil communities and were enriched in several phylogenetically distinct lineages, including the Burkholderiales. This response was conserved at higher taxonomic ranks among soils, but with variation between soils observed at the sequence variant level. Some lineages were early and consistent colonizers of the fungal hyphae, while others increased in abundance over time, indicating either a successional dynamic in the hyphosphere or growth response to the presence of fungal mycelium.

These data provide a unique profile of bacterial communities associating with extraradical hyphae in soil and can serve as the foundation for future investigation of their function and impact on the plant-fungus symbiosis.

11:00 - 11:15

EFFECT OF DARKSIDEA SPECIES ON BOUTELOUA GRACILIS: AN OVERVIEW OF THE MUTUALISM-PARASITISM CONTINUUM.

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Dark septate endophytes include dominant root-colonizing fungi of dryland plants. Interactions between these fungi and their hosts may provide drought resistance and protection against pathogens. However, adverse effects on host fitness have been recorded as well. Dark septate fungi in the genus *Darksidea* have a broad distribution across the south central United States and have been isolated from many species of grasses.

However, their roles in grass development and growth remains to be characterized. This research compared different *Darksidea* species effects on the development of *Bouteloua gracilis* (blue grama grass, Poaceae). Seventy-seven *Darksidea* isolates were recovered from grass roots collected in the south central US and were clustered into eight taxonomic operational units (OTU) based on a 97% similarity of the ITS region of the rRNA gene. Most of these *Darksidea* isolates were recovered from blue grama, black grama (*B. eriopoda*), or buffalo grass (*B. dactyloides*) from Texas and New Mexico. *Darksidea* were morphologically variable in culture. Representative isolates from each OTU were grouped into multiple clades based on comparisons of their ITS region with curated databases; the Ribosomal Database Project and UNITE. Five *Darksidea* clades were closely related to previously described species and one potentially novel species was identified. Representative isolates of each OTU were used for plant-fungal bioassays with non-sterile and surface-sterilized blue grama seeds. We recorded plant germination, tiller length, tiller number, and number of roots during plant development as well as fungal root colonization using light microscopy and scanning electron microscopy. The most mutualistic isolate increased by 9% root length in blue grama, whereas the most parasitic isolate decreased plant growth by 31%. In conclusion, our results suggest a broad range of complex symbiotic responses between *Darksidea* and blue grama.

11:15 - 11:30

BIDIRECTIONAL NUTRIENT FLUXES IN TRIPARTITE INTERACTIONS OF MEDICAGO TRUNCATULA ARE CONTROLLED BY PLANT NUTRIENT DEMAND

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The limited availability of nitrogen and phosphorus in the rhizosphere causes significant reductions in plant growth and development. When plants form mutualistic associations with beneficial microorganisms in the rhizosphere, their access to mineral nutrients improves dramatically. Arbuscular mycorrhizal (AM) fungi and nitrogen-fixing rhizobia bacteria are two dominant microorganisms in the rhizosphere. Legumes can simultaneously associate with both, thus forming a complex tripartite interaction in which the host plant, depending on its nutrient demand status, invests up to 50% of its photosynthetically derived carbon. Plant-derived carbon serves as an important driver for symbiotic functioning in the rhizosphere, but our current understanding of the mechanisms that control carbon fluxes to different root symbionts are limited. As such, we examined how plant nutrient demand and fungal access to nutrients affect carbon allocation in tripartite interactions of *Medicago truncatula* with AM fungi and rhizobia. We found that tripartite interactions led to synergistic growth responses and stimulated the uptake of phosphate and nitrogen by the plant. Both plant nutrient demand and fungal access to nutrients significantly affected carbon transport; in general, the plant

allocated more carbon to rhizobia when nitrogen was limited, but more carbon to the fungus when nitrogen was accessible. These changes in carbon allocation correlated with the expression pattern of several plant transport proteins from the Sucrose Uptake Transporter (SUT) and Sugars Will Eventually be Exported Transporter (SWEET) families, thus providing some insight into the mechanisms controlling carbon allocation in legume tripartite interactions. Additional studies demonstrated how competition among these root symbionts affects symbiotic functioning in tripartite interactions. These ongoing studies seek to further delineate both the mechanisms and the timing of shifts in carbon allocation from one symbiont to the other during tripartite interactions. A better understanding of these processes is critical to maximize nutritional benefits for legumes in agricultural systems.

11:30 - 11:45

TOWARDS THE EFFECTIVE BREEDING AND DEVELOPMENT OF IMPROVED MICROBIAL INOCULA FOR BETTER CASSAVA YIELDS IN AFRICA

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There is currently much focus on using beneficial soil microbes to improve crop yields or protect crops against biotic and abiotic stresses. But very few, if any, of these, have proven effective for globally important crops such as cassava at a large scale. Plant breeders, and before that, farmers themselves, have used naturally occurring genetic variation in crop ancestors to successfully breed greatly improved crop varieties – one of the main technological drivers of the green revolution. In contrast, proponents of microbial inoculants typically isolate one particular microbe individual, show that it improves plant growth in “lab” conditions and then expect this inoculant to be effective in real farming conditions. It is unsurprising that there have been so many disappointments.

In a joint Swiss, African and Colombian investigation in Tanzania and Kenya we have been using genetic variation in arbuscular mycorrhizal fungi (AMF) to improve cassava production. In a series of field trials we demonstrate that: 1. Inoculation of cassava with generically variable lines of the fungus can lead to extremely large differences in cassava yield in field conditions; 2. That variation in growth response could be the result of multiple factors of fungus and host plant genetics, and the environmental conditions of the planting sites.

Our work shows that there is a huge potential to use mycorrhizal fungi to greatly improve cassava production, but that this still requires a large-scale set of trials to test which are the most responsive cassava varieties and in which soils they will be most effective.

11:45 - 12:00

THE BATTLE BETWEEN ELITE RHIZOBIAL INOCULANTS AND RABBLE OF RESIDENTS AFFECTING FIELD PEA NITROGEN NUTRITION

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Rhizobial nitrogen fixation is an important driver of soil fertility in legume cropping systems and many Australian soils are lacking effective resident rhizobial associations. Success when introducing a commercial inoculant can be limited by the environmental constraints of the field and by naturalized resident rhizobial populations that may outcompete inoculants during nodulation or occupy nodules also colonised by inoculant strains. Consequences of interactions of multiple rhizobial infections on the performance of legume host plants are poorly understood. To further exploit the rhizobial N benefits in field pea systems, we looked at the effects of inter-strain interactions between a commercial rhizobial inoculant (WSM 1455) and resident strains of *Rhizobium leguminosarum* and whether these interactions were impacted under low moisture conditions (~40% less than well-watered condition) and in two different pea cultivars ('Twilight' and 'Wharton'). Our analyses demonstrate that the commercial inoculant on its own formed fewer, larger nodules and increased nitrogen fixation, resulting more nitrogen in plant tissue. In contrast, in the presence of residents, the commercial inoculant formed more, smaller nodules that fixed less nitrogen and resulted in less plant nitrogen overall. Though, there were differences for most response variables when comparing between cultivars or between watering treatments, we observed few significant interactions between these factors and the inoculum treatment. We did observe that plant biomass was reduced when the commercial and resident strains were inoculated together (compared to when inoculated individually) under well-watered conditions, but plant biomass was similar in all inoculation treatments under water stress. Taken together, our work revealed that inoculant effectiveness can be reduced as a result of competition with resident rhizobia even when inoculation leads to a high frequency of colonisation by the inoculated strain. Strain selection may be improved by assessment of inoculum effectiveness beyond nodulation success.

Session Chairs: Bobbi Helgason & Navid Bazghaleh

10:00 - 10:15

WHEAT RHIZOSPHERE MICROBIAL BIOMASS, COMPOSITION AND ENZYME ACTIVITIES IN RESPONSE TO GLYPHOSATE IN THE CANADIAN PRAIRIES

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The increasing use of glyphosate in the Canadian prairies is raising concerns about possible unintended consequences, including those on soil microorganisms. A field experiment was conducted at five sites in Alberta (2), Saskatchewan (2) and Manitoba (1) to test the impact of glyphosate applied at 0 (control), 1x, 2x, 4x and 8x the typical application rate (1 kg ae ha⁻¹), twice per season, on agroecosystem productivity and function. At the end of the four-year experiment, soil microbial biomass C (MBC), the diversities and relative abundances of bacterial and fungal communities, and the activities of β -glucosidase, β -N-acetylglucosaminidase (NAGase) acid phosphomonoesterase and arylsulphatase enzymes in the rhizosphere of wheat were assessed. Residues of glyphosate and aminomethylphosphonic acid (AMPA), the main product of glyphosate degradation, were also quantified. Large site differences were observed in most of the soil microbial properties. Concentrations of AMPA soil residues increased with increasing glyphosate application rates, but there were no glyphosate effects on most of the soil microbial properties. The few significant responses had cubic (wavy) patterns, i.e., a decrease up to a certain rate followed by an increase and then another decrease. Thus, at the typical (1x) rate, glyphosate reduced MBC and NAGase activity relative to the control, but higher rates reversed this effect. At fungal phylum level, the relative abundance of *Basidiomycota* fungi was reduced by the 2x rate relative to all other rates except the 8x rate. At fungal genus level, *Trichoderma* spp. (from *Ascomycota* phylum) were enriched in wheat rhizosphere at the 4x rate relative to other rates except the control, mainly at the two Saskatchewan sites. These responses to glyphosate are probably due to the opposite effects of glyphosate as a toxic pesticide and as a C substrate for microbial metabolism. The weeds killed by glyphosate also add C to the soil.

10:15 - 10:30

MICRO-HYDROLOGICAL NICHES IN SOILS: HOW MUCILAGE AND EPS ALTER THE BIOPHYSICAL PROPERTIES OF THE RHIZOSPHERE AND OTHER BIOLOGICAL HOT SPOTS

P Benard^{1,2}, M Zerbanadkouki¹, M Brax³, R Kaltenbach³, I Jerjen⁴, F Marone⁴, E Couradeau^{5,6}, V Felde⁷, A Kaestner⁸, A Carminati¹

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Plant roots and bacteria are capable of buffering erratic fluctuations of water content in their local soil environment by releasing a diverse, highly polymeric blend of substances (e.g. extracellular polymeric substances and mucilage). Despite this concept is well accepted, the physical mechanisms by which extracellular polymeric substances and mucilage interact with the soil matrix and determine the soil water dynamics remain unclear. High-resolution X-ray computed tomography revealed that upon drying in porous media mucilage (from maize roots) and extracellular polymeric substances (from intact biocrusts) form filaments and two-dimensional interconnected structures spanning across multiple pores. Unlike water, these structures connecting soil particles did not break up upon drying, which is explained by the high viscosity and low surface tension of extracellular polymeric substances and mucilage. Measurements of water retention and evaporation with soils mixed with seed mucilage show how these one- and two-dimensional pore-scale structures impact macroscopic hydraulic properties: i.e. they enhance water retention, preserve the continuity of the liquid phase in drying soils and decreases vapor diffusivity and local drying rates. In conclusion, we propose that the release of viscous polymeric substances and the consequent creation of a network bridging the soil pore space represent a universal strategy of plants and bacteria to engineer their own soil micro-hydrological niches where stable conditions for life are preserved.

10:30 - 10:45

ROOT SYMBIONTS AND SOIL SUCCESSIONAL STAGE INFLUENCE PLANT-SOIL FEEDBACK EFFECTS ON VEGETATION SUCCESSION

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During recovery of disturbed sites, vegetation succession can be facilitated by plant-soil feedbacks in which plants have abiotic and biotic influences on soil properties that subsequently affect plant performance. Feedbacks can be negative (likely due to pathogen accumulation) and break dominance of a plant species, or positive and promote ecosystem stability. Yet, the magnitude and direction of plant-soil feedbacks in heavily disturbed areas undergoing primary succession have not been characterized. We investigated how seedlings establishing in mine reclamation substrates versus forest soil affected establishment of conspecific and next successional stage species.

In a greenhouse, first generation willow and spruce seedlings were grown for six months in overburden (glacial till), mine tailings and forest soil. Conspecific seedlings as well as next stage successional species (spruce and western redcedar for willow; western redcedar for spruce) were grown for six months in the rhizosphere soil from the first generation seedlings and in control soil.

Overall, first generation seedlings had a negative effect on second generation seedling aboveground biomass, but feedbacks were strongest in forest soil with strength decreasing along a soil quality gradient with minimal

to neutral feedbacks in tailings. This may be because other plant growth limitations in undeveloped soils overshadow plant-soil feedback effects and/or due to a lessened capacity of the early successional ecosystems to support soil organisms (pathogenic or mutualistic).

Inter-species negative feedbacks generally corresponded with decreased mycorrhizal colonization (ectomycorrhizae in spruce and arbuscular mycorrhizae in cedar) suggesting that root symbionts can mediate plant-soil feedbacks, possibly through loss of compatible mycorrhizae. This highlights that restoration species selection can influence successional trajectories and that planting species forming compatible belowground microbial associations could improve vegetation establishment.

10:45 - 11:00

THE DESIGN AND FUNCTION OF THE RHIZOSHEATH

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The biophysical characteristics of the soil-root interface determine the efficiency of resource acquisition by the plants. We hypothesized that the pore geometry and the wettability of rhizosheath allows drought tolerant chickpea to uptake water under conditions of water stress. To test the hypothesis, we grew drought sensitive and tolerant chickpeas in soil under well-watered (90% of field capacity) conditions for 7 days and then dried the soil to conditions of 50% of field capacity over 7 days. We measured the pore geometry of soil-root interface using X-ray microtomography and destructively collected rhizosheath mass after scanning. We observed a rhizosheath mass 17% higher in tolerant than sensitive chickpea. The rhizosheath porosity of tolerant chickpea was significantly higher than porosity in the bulk soil ($P < 0.05$), whereas the porosity of rhizosheath of sensitive cultivar was significantly lower than the bulk soil ($P < 0.05$). The rhizosheath of tolerant chickpea had 28% higher pore connectivity and more macro-pores (70-110 μm) compared to the bulk soil ($P < 0.05$). Such differences were not evident in sensitive cultivar. The sorptivity (-2 cm water head) of rhizosheath soil was statistically similar in both cultivars. The stomatal conductance ($\text{mol water m}^{-2} \text{ sec}^{-1}$) of drought tolerant chickpea was ~33% higher under water stress than the sensitive chickpea and the stem water potential was 2 times higher in tolerant compared to sensitive chickpea. The stomatal conductance was 11% higher in sensitive than tolerant chickpea under conditions of no water stress. The results clearly indicated that the increased water uptake by the drought tolerant variety under water stress conditions was associated with modifications of the rhizosheath pore geometry.

11:00 - 11:15

HUNTING FOR ELITE RHIZOBIUM STRAINS TO MAXIMIZE BIOLOGICAL N FIXATION BY WHITE CLOVER

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Forage legumes such as white clover are an important component of pasture, as they not only increase feed quality, but play a key role in increasing soil fertility by providing biologically fixed nitrogen (BFN) via their symbiosis with rhizobia. New Zealand (NZ) farmers have recognised these benefits and historically inoculated rhizobia onto white clover seeds. Because of this, it has been assumed that white clover rhizobia are now naturalised in NZ soils and further inoculation of white clover will provide little benefit. Field surveys were conducted to determine whether rhizobia present in soils provide sufficient BFN for clover growth. Nitrogen fixation capacity (effectiveness) of the rhizobia in 26 soils collected across NZ ranged from 14 to 143% compared with the commercial strain *R. leguminosarum* bv. *trifolii* TA1. The number of rhizobia also varied between sites, ranging from <100 to 10⁸ colony forming unit per g soil. Large spatial variability of rhizobia within a farm (2 - 143% of TA1 across 7100 ha) and even paddocks (4 - 135% of TA1 across 0.8 ha) was detected. Spatial patchiness of rhizobia suggests that naturalised rhizobia may not be able to provide sufficient BFN to support the optimal growth of white clover. Therefore, elite rhizobia strains that can meet the N demand of modern white clover cultivars is needed. Diverse rhizobia strains (assessed by genotype by sequencing) were isolated from nodules of white clover. The symbiotic potential of >90 isolates was significantly greater than the commercial strain TA1. Meanwhile, a rapid assay for nodule occupancy has been developed to understand and screen for competitive strains which can outcompete less effective rhizobia naturalised in soils. Field trials with some elite strains has been established and ongoing. This research aims to improve BFN of white clover – the most sustainable source of N for agriculture.

11:15 - 11:30

KNOW YOUR NEIGHBOUR: HOW NEIGHBOUR ROOTS DILUTE SOIL-BORNE FUNGAL DISEASE AMONGST SUSCEPTIBLE HOSTS

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Plant diversity positively affects ecosystem functioning, but the underlying mechanisms remain debated. Recent experimental studies suggest that plant diversity may enhance ecosystem functioning by mitigating the negative effects of soil-borne fungal pathogens. This 'pathogen dilution' effect in mixtures has traditionally been explained by reduced host abundance in more diverse communities, but it has now become clear that neighbourhood composition is at least as important. Yet, we do not know how this works.

We investigated the effects of neighbours on transmission of the soil-borne plant fungal pathogen *Rhizoctonia solani* in two host species: the forbs *Leucanthemum vulgare* and *Plantago lanceolata*. We followed disease transmission through rows of host plants with and without neighbours. We compared effects on disease transmission of four neighbour species: the two hosts and two non-host grasses (*Festuca rubra* and *Anthoxanthum odoratum*). An additional factor was neighbour age: neighbours were either the same age or 10 days older than the host plants.

We found that *R. solani* disease transmission was significantly higher in the host *P. lanceolata* compared to the host *L. vulgare*. Moreover, we found significant effects of both neighbour-species and –age on disease transmission. The only same-age neighbour species that affected disease transmission was *P. lanceolata*, which increased disease transmission in *L. vulgare* hosts. Amongst the 10-day older neighbour species however, *L. vulgare* decreased disease transmission in both host species and the two non-host grasses decreased disease transmission in *L. vulgare*, but not in *P. lanceolata*.

Our findings provide proof of principle that plant neighbour identity affects belowground fungal disease transmission. Furthermore, these results support neighbour species- and age-specific root-mediated mechanisms of soil-borne disease amplification and dilution. Future research will focus on understanding the potential physical, chemical, and microbiological mechanisms underlying these effects of neighbours on disease risks in diverse plant communities.

11:30 - 11:45

BIOTIC VERSUS ABIOTIC DRIVERS: DISENTANGLING KEY FACTORS SHAPING RHIZOSPHERE PHOSPHORUS SPECIATION ALONG A CLIMATE GRADIENT

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Phosphorus is a key element for the cellular energy cycle and an essential constituent of cell walls, DNA and RNA. Microorganisms and plants developed strategies to utilize phosphorus from organic and inorganic sources, such as expression of extracellular phosphatases and excretion of low-molecular-weight organic acids.

With this study we aim to investigate phosphorus speciation in dependence on climatic conditions, soil depth and root distance.

Samples were taken from three sites, all of which have developed on granodiorite along the Chilean-Coastal-Cordillera, covering a climatic gradient from semi-arid over Mediterranean to humid-temperate conditions. To study changes in phosphorus speciation vertically and from rhizosphere to bulk soil, we took samples in three depth and in millimeter increments from roots to non-rhizosphere soil. Phosphorus speciation was determined by Synchrotron-based X-ray absorption near-edge structure spectroscopy. Contents of low-molecular-weight organic acids, phosphatase activity, substrate affinity and microbial biomass carbon, as total bacterial and fungal DNA, were measured.

For the precipitation gradient and soil depth we found decreasing contents of primary phosphorus minerals with increasing precipitation and soil depth. Rhizosphere soil was depleted in sesquioxide-adsorbed inorganic phosphorus and apatite, while organic phosphorus increased in comparison to bulk soil. In the semi-arid site, the content of low-molecular-weight organic acids was strongly correlated with the amount of primary minerals, indicating that exudation of organic acids serves phosphorus mobilization and, hence, intense biotic weathering. The site under humid-temperate conditions reveals a strong negative correlation of microbial biomass carbon and phosphatase activity with inorganic phosphorus adsorbed to sesquioxides, allowing to infer that P nutrition in this ecosystem relies less on biotic weathering of phosphorus minerals, but mainly on organic sources. With this study we show that ecosystem phosphorus nutrition strategies and crucial biotic and abiotic factors controlling phosphorus speciation can be identified by assessing rhizosphere- versus root free bulk-soil.

11:45 - 12:00

ACTIVE FOOD WEB COMPONENTS IN GRASSLAND RHIZOSPHERE NETWORKS DURING NATURE RESTORATION

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Soil organisms have an important role in aboveground community dynamics and ecosystem functioning. However, most studies have considered soil biota as a black box or focussed on specific groups, whereas little is known about entire soil networks. Here we present results that during the course of nature restoration on abandoned arable land a compositional shift in soil biota, preceded by tightening of the belowground networks, corresponds with enhanced efficiency of carbon uptake. We discovered that although fungi represent a relative small amount of the total microbial biomass half the amount of carbon that flows from plants into soil is taken up by the soil fungi in early stages of succession. After 30 years, that share has risen to three quarters of the plant-derived carbon stored in the soil. By labelling the carbon atoms, we were able to follow the carbon flow into the soil food web. In this way, we could link the organisms to their corresponding functions in the community. We use stable isotope tracing in the DNA fraction with illumina-sequencing and the fatty acid (PLFA/NLFA) fraction for coarse level analyses to find out who is driving who during land use change. We have also actively manipulated soil cores from the field by transplanting from natural grasslands into agricultural fields with soil as well as microbes and fauna. We assessed the effect on the carbon flow and responses on the

plant community. We will be able to show preliminary data on active photosynthesized carbon that travels via root exudates through the rhizosphere community under contrasting land uses, as well as give a prospect on the wider applications for this in terms of carbon sequestration potential in these grasslands.

SESSION 22: RHIZOSPHERE OF CROPPING SYSTEMS

(GALLERY C)

Session Chairs: Kari Dunfield & Micaela Tosi

10:00 - 10:15

ORGANIC MANAGEMENT STRENGTHENS INTERKINGDOM RELATIONSHIPS IN THE SOIL AND RHIZOSPHERE OF BARLEY

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Soil microbial connections are critical determinants of aboveground diversity, but little is known about how soil management practices modify multi-trophic interactions. To gain insights on the ecological relationships between different trophic levels of soil biota, we investigated the effect of agricultural practices on soil inter-kingdom interactions in the rhizosphere and bulk soil compartments. We examined the influence of organic and inorganic fertilization, and their combinations on (i) individual groups (bacteria, protozoa and fungi as microbe representatives and metazoans as microfauna representatives) and (ii) inter-kingdom interactions (focusing on the interactions between bacteria and eukaryotic groups). The microbial communities were analyzed by sequencing ribosomal genes of metabolically active bacteria (16S *rRNA*), fungi, protozoans and microfauna (referred to here as metazoans; 18S *rRNA*). We hypothesized (i) increased complexity of bacterial interactions under organic fertilization, with fewer associations with the main primary decomposers fungi; (ii) increased associations of bacteria with the main grazers predators, protozoans and metazoans, under mineral fertilization; (iii) a stronger effect of fertilization type on the overall associations among different groups in the rhizosphere than in bulk soil; and (iv) an additional driving force of environmental factors on taxa co-occurrence feedback. Overall, organic amendment resulted in more complex interactions, with inter- and intra-connections among primary decomposers (bacteria and fungi) and predators (protozoan and metazoans) independent of soil compartment. When environmental factors of bulk soil were taken into account, the inter-connections between primary decomposers and predators became more pronounced. The changes in bacterial abundance under organic fertilization selected orders with different associations. These associations are most likely related to survival strategies and predation for protozoans and metazoans, while fungal-bacterial associations are most likely mediated by cooperation in decomposition. Our findings support that organic fertilization offers a more complex environment than inorganic fertilization in driving multifaceted multi-trophic strategies.

10:15 - 10:30

THE RHIZOSPHERE MICROBIOME: HOW BIODIVERSITY AND PLANT BREEDING CAN SHAPE FUNCTIONAL TRAITS IN RESPONSE TO BIOTIC/ABIOTIC STRESSES

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The rhizosphere microbiome impacts on plant growth, development and resistance against soil-borne pathogens. Plants shape their rhizosphere microbiome composition through exudates that stimulate or repress specific microbial genera that may provide a range of ecosystem services such as nutrient acquisition and abiotic stress tolerance. We investigated the composition and metabolic potential of the rhizobacterial community of different common bean (*Phaseolus vulgaris*) cultivars with variable levels of resistance to the fungal pathogen *Fusarium oxysporum* (Fox). For the different bean cultivars grown in two soils with contrasting physicochemical properties and microbial diversity (agricultural soil, Amazonian Dark Earth - ADE), rhizobacterial abundance was positively correlated with Fox-resistance. Pseudomonadaceae, Bacillaceae, Solibacteraceae and Cytophagaceae were more abundant in the rhizosphere of the Fox-resistant cultivar. Network analyses showed a modular topology of the rhizosphere microbiome of the Fox-resistant cultivar, suggesting a more complex and highly connected bacterial community. Metagenome revealed that specific functional traits such as protein secretion systems and biosynthesis of antifungal phenazines and rhamnolipids were more abundant in the rhizobacterial community of the Fox-resistant cultivar. Metatranscriptome revealed that the microbiome of the Fox-resistant cultivar presented high expression of genes affiliated to Paenibacillaceae, a group known by its antifungal activity. The Fox-resistant cultivar also presented high expression of genes related to metabolism of nutrients and specific functional traits related to pathogen suppression, such as motility and chemotaxis, and phenazine and colicinV. Network analysis showed similar results to the metagenome approach and pointed the genus *Paenibacillus* as a keystone species in the microbiome and most prevalent in soil with high microbial diversity (ADE). Our findings suggest that breeding for Fox-resistance in common bean have co-selected for other unknown plant traits that support higher abundance of specific beneficial bacterial families in the rhizosphere with functional traits that support a more complex rhizosphere microbiome provided by the ADE.

Acknowledgement: CNPq, CAPES, FAPESP

10:30 - 10:45

NITROGEN COMPLEMENTARITY UTILIZATION, BIOLOGICAL N₂ FIXATION OF SOYBEAN AND PEANUTS AND N TRANSFER TO ASSOCIATED MAIZE IN INTERCROPPING UNDER FIELD CONDITIONS

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Legumes/non-legumes intercropping are widely considered as a sustainable agricultural practice, due to yield advantage and efficient N complementary utilization and biological N₂ fixation (BNF) and nitrogen transfer from legumes to non-legumes. Previous studies in N transfer mainly was in greenhouse experiments, there are few results in situ under real field intercropping conditions. We conducted field experiments in Lishu Experimental Station of China Agricultural University, located in Northeast part of China in 2018. The field experiment was a split-plot design that main plot was N application rates (N₀, N₁, N₂, and N₃) and split-plot was five cropping systems (maize/soybean and maize/peanuts intercropping, and corresponding monocropping). The nodulation parameters, symbiotic N₂ fixation of legumes was measured by natural isotope abundance method, and N transfer from legumes to maize was determined by using soil dilution ¹⁵N method and petiole injection ¹⁵N method in N₂ treatment under field in situ conditions. Results showed that intercropped maize is dependent on more N from soils and fertilizer, whereas legumes rely more on symbiotic nitrogen fixation than corresponding monocropped maize, soybean and peanut, probably derived from interspecific N competition. Nitrogen fertilization significantly inhibits the nodulation and BNF of both soybean and peanut, while phosphorus fertilizer had no significant effect on the nodulation and BNF. We did not find significant N transfer maize/soybean intercropping, whereas peanut did transfer nitrogen to maize in maize/peanut intercropping. Nitrogen loss of intercropping systems was significant lowered relative to monocropping based on ¹⁵N methods. In summary, Intercropping did not change nodulation and BNF in soybean/maize and peanuts/maize intercropping, compared with monocropped soybean and peanuts. Nitrogen transfer from legumes to maize is dependent on associated legumes, and its contribution to N nutrition of maize is limited under the field conditions.

10:45 - 11:00

CROP DIVERSIFICATION POSITIVELY ALTERS THE INTENSITY OF SOIL-PLANT INTERACTIONS IN INTENSIVE AGROSYSTEMS

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Conservation of soil microbial diversity and plant abilities to recruit beneficial microbes is a promising lever to reduce synthetic inputs. However, such processes are partly based on functional plant diversity that determine soil microbial abundance, diversity and activities. As a consequence, crop diversification could improve plant nutrition and health through changes in ecological functions fulfilled by rhizosphere microorganisms such as disease suppressiveness or nutrients provisioning. Our aim is to identify at the field

scale if a "rhizosphere effect" can be observed in intensive farming systems in order to estimate the potential of plant-soil feedbacks. We hypothesized that (i) a Rhizosphere Effect Index (REI) can be estimated and (ii) the value of the REI will describe a gradient of crop diversification. Accordingly, we created a REI by comparing bulk versus rhizosphere variables in fields located on three farms from North West France differing by their crop diversity index (sensu Tiemann et al., 2015). Experiment was conducted from March to July 2018, and soil (rhizosphere and bulk) and plant (*Triticum aestivum*) were collected every 2 weeks to measure enzymatic activities, nitrate and phosphate fluxes estimated by in-situ anionic membranes. Plant variables (diseases damages, development stages and aerial biomass) and soil microbial diversity and abundance were also measured. The REI values, comprised between -1 et 1, significantly discriminated ($p < 0.05$, 2-ways Anova & HSD test) rhizosphere soils compared to bulk soils and allowed comparisons between the three crop diversity situations. Enzymatic activities better defined the rhizosphere effect with systematically lower rhizosphere effect for the less diversified farm. Interestingly, temporal patterns can be analysed with the REI along the crop cycle. Based on these results, we can consider that crop diversification, even in intensive farms, influence the rhizosphere effect, which is exacerbated in high diversification farms, suggesting opportunities for agroecological transitions in high diversification farms.

11:00 - 11:15

LONG-TERM FARMING PRACTICES SHAPE THE RHIZOSPHERE MICROBIOTA AND PLANT HEALTH

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Belowground plant-microbe interactions are crucial for plant development and growth. While previous studies revealed that agricultural management practices shape biotic and abiotic soil properties, we aimed here to understand their impact on the rhizosphere microbiota in order to promote a sustainable plant production. We hypothesized that the impact of long-term agricultural management on the soil microbiota is conveyed via rhizosphere microorganisms to the future plant generation affecting its performance.

We used soils from three long-term field trials established in 1978 (Therwil, Switzerland), 1992 (Bernburg, Germany) and 2006 (Thyrow, Germany) to grow the model plant lettuce (*Lactuca sativa* L.) for ten weeks under controlled growth chamber conditions. These field trials allow to study the impact of different fertilization strategies and intensities, tillage practices and crop rotation.

High-throughput sequencing of bacterial 16S rRNA genes and fungal ITS fragments amplified from total community DNA showed significant differences in soil and rhizosphere microbial community compositions depending on field sites and long-term farming practices. However, Firmicutes were enriched irrespective of the field site in the rhizosphere of lettuce grown in organically fertilized soils. When cultivated in organically fertilized soils, a higher expression of several stress-related genes was observed by RT-qPCR analysis in lettuce leaves although plants were visibly free of disease symptoms. Quantity and quality of lettuce root exudates differed among the soils under different farming practices. Moreover, differences in plant productivity and soil suppressiveness potential towards the soil-borne model pathogen *Rhizoctonia solani* were recorded depending on the site and agricultural management.

Under controlled growth chamber conditions, we could show that the lettuce rhizosphere microbiota assemblage as well as plant growth and health were significantly influenced by farming practices. Our study suggests an agricultural legacy on the establishment and performance of a subsequent plant generation and its associated rhizosphere microbiota.

11:15 - 11:30

FUNTIONAL ROOT TRAIT BASED CLASSIFICATION OF COVER CROPS TO SUPPORT SUSTAINABLE AGRICULTURE

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The use of cover crops (CCs) is a well-established soil conservation technique and has been effectively used for soil erosion control in many arable systems. Whereas the obvious protection mechanism of CCs occurs through canopy protection of the soil, plant roots provide multiple important functions. It is important to consider the soil functions delivered by different root systems in order to increase the impact of CCs on sustainable soil and water management. A classification of CC root systems up to 0.5-1m deep based on functional traits is needed to study their soil bio-engineering purposes such as soil structure improvements for hydrological services and optimised crop growth. To do so, a greenhouse experiment is set-up using large containers (1m x 1.2m x 0.8m) filled with sandy clay loam in which 7 cover crop species (oat, rye, buckwheat, vetch, radish, mustard and phacelia) were grown for 90 days. In situ root images were taken weekly using mini-rhizotrons and root cores were taken at the end of the experiment. Those roots were washed and imaged on a flat-bed scanner with WinRhizo software. Following root traits were determined: topsoil and subsoil root surface area, topsoil and subsoil root length density, topsoil and subsoil average root diameter, topsoil and subsoil root specific length, deep root mass fraction, topsoil and subsoil fine root length fraction and length of the root hairs. For each species treatment, root identity is determined from a distinctive combination of single root traits using PCA. The PCA coordinates of each species on the two main principal axes (PC1 and PC2) are then used to calculate a multidimensional root functional identity (RFI) and root functional diversity (RFD) index for each species. Multidimensional statistics (linear mixed models) are used to relate RFI and RFD to the different soil functional variables.

11:30 - 11:45

ENGINEERING ROOT/RHIZOSPHERE PROCESSES FOR IMPROVING NUTRIENT-USE EFFICIENCY AND YIELD IN CHINESE INTENSIVE MAIZE-CROPPING SYSTEMS

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Rhizosphere dynamics have been widely investigated since the beginning of last century but little attention has been paid to the process-based rhizosphere management and engineering at an agroecosystem level. Plants can regulate root morphological traits, and modify rhizosphere processes through physiological activities, particularly the exudation of organic acids, phosphatases, and protons. However, the underlying strategy of root/rhizosphere processes and management in intensive cropping systems with high input of fertilization remains largely to be determined despite advances in rhizosphere research. Here, we examined how to maximize the efficiency of root growth and rhizosphere processes by designing localized nutrient supply and rhizosphere interactions, and evaluated the contribution of rhizosphere management to nutrient-use efficiency and maize yield. Maize was treated with localized application of P with NH₄-N, NO₃-N or urea. The density of lateral roots in local nutrient patches increased by 50%. Maize yield increased by 10%, and agronomic N and P efficiency increased by 40% and 80%, compared with the control. Localized supply of P plus NH₄-N enhanced root proliferation and rhizosphere acidification, resulting in the high activity of acid phosphatases in the rhizosphere, which significantly improved nutrient uptake and maize yield. Our findings highlight that plant roots can coordinate root morphology, root physiology and rhizosphere processes to maximize soil nutrient acquisition and use through engineering root-zone nutrient supply and rhizosphere interactions. Localized application of P plus NH₄-N stimulated nutrient uptake and plant growth by integrating root proliferation and physiological traits involving increased proton release, acid-phosphatase activity and expression of ammonium transporters. Moreover, maize yield was further enhanced by intercropping with faba bean due to rhizosphere interactions. It is suggested that engineering root/rhizosphere processes through optimizing nutrient management to “feed roots, not soils” and maximizing rhizosphere efficiency is an effective strategy for improving nutrient-use efficiency and grain yield in intensive maize production.

11:45 - 12:00

HIGH MAIZE DENSITY ALLEVIATES THE INHIBITORY EFFECT OF MINERAL N ON NODULATION OF INTERCROPPED PEA

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Symbiotic N₂ fixation is essential in the development of sustainable agriculture, but the nodulation of legumes is usually inhibited by N fertilization. Enhancing interspecific competitiveness through optimizing maize density maybe can alleviate the inhibitory effect of soil mineral N on intercropped pea and improve system performance through interspecific relations. We hypothesized that suitable maize plant density could enhance the utilization of soil mineral N in pea strip, facilitate pea nodulation and alleviate the inhibitory effect of soil

mineral N on the pea. A field experiment was conducted from 2012 to 2014 in the Hexi Corridor region of northwestern China. The experiment consisted of monoculture pea and a pea / maize strip-intercropping systems. Two N fertilization rates were evaluated in both cropping systems during the co-growth period of intercropping, 0 kg N ha⁻¹ (N₀) and 135 kg N ha⁻¹ (N₁), and three maize densities were evaluated with both levels of N fertilization in the intercropping system: 45,000 (D₁), 52,500 (D₂), and 60,000 plants ha⁻¹ (D₃). The results showed that soil mineral N of D₃ decreased 8% and 12% than that of D₂ and D₁ in intercropped pea strip, alleviation of the inhibitory effect on nodulation in intercropped pea (C_a) increased by 2.9 and 18.1 fold accordingly, and NUE of the pea improved by 6% and 9% under the N application, respectively. This suggested that optimizing maize plant density in maize/pea intercropping system can regulate soil mineral N uptake, alleviate the inhibitory effect of soil N on nodulation of the pea, and improve nitrogen use efficiency.

12:10 - 13:30 CLOSING CEREMONY / STUDENT POSTER AWARD / RHIZOSPHERE 6 ANNOUNCEMENT

POSTER LISTING

P.01

NEW INSIGHT INTO THE MICROBIOME OF THE TERMITE MOUND AND ITS SURROUNDING SOILS

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Termite mounds are hotspots of nutrients and may significantly influence the soil dynamism in terms of nourishment and fertility. The microbial communities influencing the nutrients in the termite mound are poorly understood. Termite microbiome studies so far have focused on the gut symbionts, with limited studies on microbiome of the termite mounds. Hence, we conducted 16S amplicon sequencing of termite mounds and the surrounding soil to begin to characterize the microbiota influencing the nutrient distribution in these mounds. This study showed that the microbial communities of the termite mound are much richer than the non-termite soil. There is a very high significance differences of $p\text{-value} < 0.0001$ in the bacterial community composition between different non-termite and termite mounds through the beta diversity statistical analyses. The comparative studies using alpha diversity studies revealed similarities within the groups of non-termites and termite mounds. Concurrently, there is an abundance of agriculturally useful microbial phyla identified in the termite mounds. The termite soil possessed Euryarcaeota as the most abundant Archaea and Proteobacteria, followed by Actinobacteria, as the most predominant phyla among the Bacteria in the mound consortia. The taxa Betaproteobacteria, Oxalobacteraceae, Burkholderiales, Massilia, and Niabensis were the most abundant class, family, genus, order, and species, respectively. Conversely, in the non-termite soil, the most abundant Archaea and Bacterial phylum as Thaumarchaeota and Verrucomicrobia respectively. Termite mounds consist of previously unknown species than the non-termite, an indication of greater ecological diversity. The significant phyla discovered in the mound are of great influence on soil health and fertility, although greatly influenced by the environmental factors.

P.02

PLANT SPECIES MODIFY SOIL MICROBIAL COMMUNITY STRUCTURE OVER TIME

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Soil microbes are an essential biotic component of soils, and their functional roles (beneficial or pathogenic) can determine the health of a soil ecosystem. Terrestrial plants, with their root systems, interact strongly with the soil and soil microbes and have been suggested as one of the strongest determining factors of microbial community assembly. Current evidence suggests that different plant species can select for different soil microbial communities around their roots, but it is difficult to weigh this evidence across studies because of the various methods used in different studies (e.g. sampling time, location, soil type, and microbiological analysis

tools). At the same time, despite continuous mono-cropping being broadly implemented in various agricultural systems, the understanding of how these systems may develop disease suppressive characteristics remain limited for annual crops. To address these challenges, we conducted a controlled field experiment that allowed us to directly compare soil microbial community structure and function under different plant species over time in a tropical oxisols soil. We will discuss fungal and bacterial richness, community changes that occurred under different plant species, the network of bacteria and fungi, as well as the interactions among these two groups of soil microbes among different plant species and over time.

P.03

GARLIC STALK- ARBUSCULAR MYCORRHIZAL FUNGI MEDIATED SYNERGISTIC INTERACTION SUSTAINED THE SOIL ENVIRONMENT THROUGH REGULATION OF BACTERIAL COMMUNITY COMPOSITION AND DIVERSITY STRUCTURE IN AN ANTHROPOGENIC CONTINUOUS CUCUMBER PLANTED SOIL.

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The escalated negative soil-plant feedback due to long-term continuous cropping obstacles could be attenuated by adopting sustainable and cost-effective agronomic measures. However, underlying mechanism of crop productivity and soil biota structure through compatible soil organic inputs is less understood under intensive vegetable production system. We conducted consecutive greenhouse experiments (2016-2018) to study the legacy/priming effects of soil amendments through addition of indigenous organic substrate (GS: garlic stalk) and mycorrhizal inoculants (AMF: *Glomus versiforme* L.) in a continuous replanted soil. We found that repeated application of co-amended GS+AMF treatment significantly induced the soil quality changes by increasing organic matter, soil nutrient contents and biological activity and thus cucumber yield. The additive effect of GS amendment unveiled the structural development of AMF characteristics; trigger the mycorrhizal strength, sporulation, extend the hyphal growth and facilitate the mutualistic coordination to acquired host-compatible symbiosis. Our 16S rRNA microarray coupled with PCA and NMDS analysis revealed the distinctive role of organic amendments in shaping the community structure and composition. The bacterial diversity is more conducive and sensitive to sole organic substrate, delineated the highest richness and diversity indices under GS amended soil. Redundancy analysis suggested that the predominant taxa was influenced: directly by the GS, AMF, GS+AMF applied, and indirectly by soil edaphic variables. Soil pH with biological activation was the important predictor for structure of the soil bacterial community in our cropping system. The predictive metabolic characteristics (metabolism, environmental information processing, genetic information process) associated with host-plant symbiosis could be an important ecological signals that provide direct evidence of a community's functional capabilities. Results from the present study suggest a well-adopted garlic-AM symbionts in this cultivation system, and the consequent positive synergistic interaction is the key mechanism of induced soil quality environment which ultimately can promote the seasonal crop yield and mediate soil microbial activity.

P.04

ANALYSIS OF THE FUNCTION OF SANTHOPINE, AN AMADORI-TYPE OPINE, IN TOBACCO RHIZOSPHERE

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Agrobacterium transfers the T-DNA to the host plant cells. T-DNA expression induces the formation of crown gall tumor or hairy root and the synthesis of amino acid derivatives, called opines. Santhopine is an Amadori-type opine synthesized by the conjugation of glucose and glutamine, which is catalyzed by santhopine synthesis enzyme encoded by *mas2* gene. Recent work has shown that the genome of *Nicotiana tabacum* contains *mas2* gene acquired from *Agrobacterium rhizogenes* by horizontal gene transfer. The expression of *mas2* and santhopine accumulation were detected in leaves and root of *N. tabacum* cv. Burley 21. It has been reported that several bacterial species such as *Arthrobacter* possess santhopine catabolism genes in addition of *Agrobacterium* spp., suggesting various roles of santhopine in plant-microbe interactions in the rhizosphere. To elucidate the function of santhopine metabolism in the tobacco rhizosphere, the effects of santhopine on the soil bacterial communities are investigated.

In order to investigate the effects of santhopine on the soil bacterial populations, three different concentrations of santhopine solutions were added in the soil and the bacterial communities were assessed by 16s rRNA gene amplicon sequencing. Observed-OTUs and Shannon index decreased along with santhopine concentration. PCoA analysis using Unifrac distance indicated that it forms different community composition depending on the santhopine concentrations. Furthermore, the relative abundance of Microccaceae correlated with the santhopine concentration, suggesting that bacteria belonging to Microccaceae utilized santhopine as an energy source. We then isolated soil bacteria from santhopine treated soil and conducted santhopine degradation assay. It was shown that *Arthrobacter*, belonging to Microccaceae, showed santhopine degradation. *Arthrobacter* was isolated from tobacco root endosphere and most of *Arthrobacter* isolates showed santhopine degradation ability as well. These results suggest santhopine is a specific energy source for *Arthrobacter* and play an important role for the recruitment of *Arthrobacter* to tobacco rhizosphere or endosphere.

P.05

ROOT ENDOPHYTIC SERENDIPITA SPECIES AFFECT TOMATO CULTIVAR-SPECIFIC RESPONSES TO FUSARIUM WILT

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Endophytic fungi from the family *Serendipitaceae* colonize a vast amount of different plant species and have recently gained increasing attention due to their growth-promoting and bioprotective properties. The majority of studies were conducted with the most prominent representatives, such as *Serendipita indica* and *S. vermifera*. However, little is known about other representatives, such as *S. williamsii* and *S. herbamans*. To test their biocontrol and growth-promoting potential, a susceptible and tolerant tomato cultivar (Kremser Perle and

Micro-Tom, respectively) was inoculated with *S. williamsii*, *S. herbamans*, *S. indica* and *S. vermifera* and challenged with the soilborne pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) in greenhouse experiments over two seasons. Furthermore, *in vitro* assays on the direct inhibitory effects of *Serendipita* spp. against *Fol* were performed. Here, we show for the susceptible cv. Kremser Perle that the negative effects of *Fol* on plant biomass were alleviated by all four applied *Serendipita* spp. 56 days after inoculation. Apart from these similar effects on biometric parameters, the impact on disease incidence differed. Hence, *S. herbamans* and *S. vermifera* reduced the disease incidence by almost 50% compared to the *Fol* treatment. In the tolerant cv. Micro-Tom, disease incidence and disease severity were not affected by the application of *Serendipita* spp. However, shoot dry mass was negatively affected by *S. vermifera* under *Fol* disease stress. *In vitro* assays did not reveal any direct inhibitory effects against *Fol*. In conclusion, *S. herbamans* proved to be an effective antagonist against *Fol* in the two investigated cultivars, which also implies a still unexplored potential of putative future isolates.

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P.06

TOMATINE SECRETION FROM TOMATO ROOTS AND ITS EFFECT ON THE BACTERIAL COMMUNITY

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Tomato (*Solanum lycopersicum*) contains α -tomatine, which is composed of a C₂₇ steroid tomatidine with an oligosaccharide, in most plant tissues. Tomatine is known to be toxic against various organisms. However, dynamics and functions of tomatine in rhizosphere are still unknown. Here, we investigated the secretion of tomatine from tomato roots both in hydroponic culture and field condition as well as its functions in the rhizosphere. First, to characterize accumulation and secretion of tomatine during tomato development, we quantified tomatine in leaves, roots, and root exudates of tomato plants during the period from 3 to 13 weeks old. Tomatine secretion per plant peaked at 7 weeks old, while the secretion per root fresh weight peaked at 3 weeks old. In accordance, roots of 3 weeks old accumulated the highest tomatine contents per fresh weight. By contrast, few changes of the contents in leaves throughout plant development was observed. The secretion of tomatine was also analyzed in tomato field. To evaluate the fate of tomatine in the rhizospheres, we incubated authentic tomatine with the soils at 28°C during the period from 0.5 to 7.0 d, and then analyzed the tomatine and degraded compounds using LC-MS. Tomatine was degraded from 0.5 d after incubation, while tomatidine increased from 0.5 d to 2.0 d after incubation. The degradation rate constant for tomatine was determined to be 1.3 d⁻¹. To analyze the effect of tomatine on the bacterial community, we applied authentic tomatine into the field soils and incubated at 28°C for 15 d. The 16S rRNA amplicon sequence analysis of each soil DNA was then performed. Changes of bacterial communities were observed after 15 days, with highly abundant genus in tomatine-treated soil.

P.07 (will also be presented as poster pitch)

ATTACHMENT AND GROWTH ON THE RHIZOPLANE: QUANTIFYING ROOT/BACTERIAL DYNAMICS

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Colonisation of the root surface, or rhizoplane, is the first step towards becoming established in a plant's microbiome for beneficial, commensal and pathogenic soil born bacteria. When and where bacteria first colonise the rhizoplane has a large impact on the success of a strain in the mature rhizosphere microbiome, yet comparatively little is known this process. Colonisation is, with factors such as bacterial attachment and proliferation playing key roles. Previous studies of soil microbial dynamics have viewed the rhizoplane as static, as a result, they have failed to fully explain observed shifts in microbial composition taking place when roots colonise new regions of soil. This study aims to describe and quantify the early stages of rhizoplane colonisation, viewing the root as a dynamic system.

The first step towards achieving this aim has been quantifying, and distinguishing between, bacterial attachment to, and proliferation on, the rhizoplane. A model system of Lettuce (*Lactuca sativa*) and fluorescently tagged *Pseudomonas fluorescens* SBW25 has been used in a combination of microbiological assays and confocal imaging. In this system, bacteria have been shown to follow a replicable pattern of colonisation beginning with a period of attachment after which proliferation on the root surface becomes the dominant factor in increasing bacterial numbers. Bacteria have been shown to be reliant on the presence of the root for proliferation. These methods have potential use for studying the colonisation patterns of other organisms, including plant growth promoters and plant/animal pathogens. Going forward; the challenge will be to investigate other important aspects of microbial establishment on root surfaces, such as bacterial chemotaxis. This will show how dynamic plant and microbial traits can be used to explain observed shifts in the rhizosphere community, and understand the key stages of colonisation.

P.08

RESPONSES OF RHIZOSPHERE NEMATODE COMMUNITIES TO DIFFERENT PLANT FUNCTIONAL TYPE IN FALLOWED RED SOIL

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Plants play pivotal roles in resource input of terrestrial ecosystem. Ecologists are increasingly adopting trait-based approaches to understand how community change influences ecosystem processes. However, most of research work have ignored root traits, which are the important drivers of many ecosystem processes, such as composition and changes of soil biota. Soil nematodes occupy a central position in soil ecosystem. Despite different plant species could have different root trait, which change rhizosphere soil conditions and soil community, however, there is little awareness on the inter-relationship between root trait and nematode community. In order to explore response of rhizosphere nematode communities to different root traits, we

chose typical plant functional types from a fallowed red soil, including Compositae *Conyza Canadensis* (Con.), Gramineae *Paspalum natatu* (Pas.) and *Setaria viridis* (Set.), Leguminosae *Cassia rotundifolia* (Cas.) and *Lespedeza bicolor* (Les.), and collected samples of plants and rhizosphere soil at vigorous and mature stages. Our results showed that at two sampling stage, Gramineae had more nematodes than Leguminosae or Compositae. Herbivorous nematodes had the highest proportion among all trophic groups (45.2% - 77.9%). Nematode abundance was associated with root trait and rhizosphere soil properties, positively related to root biomass, root length and root surface area, while negatively correlated to root nitrogen content. Redundancy analysis (RDA) indicated that root nitrogen concentration and average surface area have strong effects on nematode communities, and there is a correlation between nematode trophic group and root carbon concentration, root biomass, and surface area. This study provides a basis for understanding the changes and controlling factors of soil food webs during the succession of plant community in the fallowed farmland field. The ecosystem structure and function remain to be further studied in order to use suitable plants for the restoration of degraded red soils.

P.09

THE ROLE OF PLANT-ASSOCIATED MICROBIOTA IN PLANT-SOIL FEEDBACK OF PRIMARY SUCCESSIONAL PLANTS

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During succession, the establishing plant community alters soil environment by rhizodeposition and organic matter input which results in shifts in soil physico-chemical properties and in the composition of microbial communities. Soil microbes in turn regulate nutrient cycling, contribute to soil formation and form mutualistic or pathogenic relationships with plants, potentially affecting the plant-soil feedback. In this study, we aimed to experimentally investigate the biotic and abiotic mechanisms underlying plant-soil feedback interactions among plants from a successional series in a limestone quarry landfill left to spontaneous development.

We established a two-phase microcosm experiment. In an initial phase, soil was conditioned with monocultures of three early- and three mid-successional plant species occurring at the landfill. In the second phase, plants from different successional guilds were grown in conditioned soil to assess the effect of conditioning on plant performance.

Both bacterial and fungal communities in soil conditioned by plants differed from control soils and also among plant species and were shaped predominantly by plant species identity per se. Changes in fungal, but not bacterial, community correlated with changes in soil chemistry and plant biomass. Together, these results suggest that individual plant species influence soil fungi predominantly through changes in soil chemistry and that this effect is proportional to plant biomass. Contrastingly, plant-species effects on bacterial community are independent of plant biomass and are only partially mediated by soil chemistry. Soil chemistry and the biomass of conditioning plants were also the most important determinants of plant-soil feedback responses of individual

plants, while soil microbes played only a minor role. Soil fungi influenced plant seedling establishment but not plant final biomass, suggesting that biotic feedbacks might be more important in the initial stages of plant life.

P.10 (will also be presented as poster pitch)

NATURALLY EXISTING AND PLANT-BENEFICIAL MYCORRHIZAL-BACTERIAL ASSOCIATIONS

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In the rhizosphere, arbuscular mycorrhizal fungi form mutualistic symbiosis with plants where they access carbohydrates from plant roots and in return, increase absorption of nutrients from the soil environment. Mycorrhizal fungi have multiple positive effects on growth and health of the plant i.e. help the plants access nutrients and resist abiotic and biotic stresses. Bacteria are very closely associated with mycorrhizal spores in the rhizosphere and associated bacteria may be the third partner involved in mycorrhizal-plant symbiosis. They may play an important role in influencing plant hosts directly or indirectly as well as interact at varying levels to enhance mycorrhizal activity, nutrient uptake, and provide resistance against various stresses. Understanding the mechanism of this natural, inter-kingdom functional co-existence in relation to host plants and mycorrhizal structures, will aid in developing new strategies for sustainable crop production and hence their mechanistic details still needs to be analysed in depth. In our study, we have screened geographically diverse species of arbuscular mycorrhizal fungi growing under both *in-situ* and *in-vitro* conditions to determine the associated bacterial diversity and their functional relevance. A wide range of bacterial strains were isolated, purified and characterized on the basis of 16S rDNA analysis. Plant growth promoting functional traits such as phosphate and potassium solubilisation, nitrogen fixation, indole acetic acid production, biocontrol potential and biofilm formation were tested and it was observed that 10 bacterial strains possessed multifarious plant growth promoting traits (at least three traits). Bacteria associated with mycorrhizal structures were confirmed to be live and in the form of clusters/aggregates through microscopic techniques. Further focus is on understanding the mechanistic aspects involved in such agriculturally beneficial, fungal-bacterial relationships with a model plant host which will allow their subsequent usage and modulation for sustainable agricultural practices.

P.11

CONTRASTING METABOLIC PROFILES FROM HYDROPONIC AND SOIL GROWN BARLEY

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Root exudates are increasingly recognised as a key driver of global carbon cycling and ecosystem function. The analysis of root exudates has traditionally taken place on plants that have been grown in hydroponic systems.

However, this environment lacks the highly complex and heterogeneous structure, as well as the microbial community, of soil, and collected root exudates might not be meaningful for predicting their function in a soil environment. As root exudates are key in plant communication with, and the function of, the rhizosphere, recent methods have focused on analysis of root exudation in predominantly soil-based systems. Such approaches enable analysis of plant metabolomic responses to environmental cues in more natural conditions. In this study plants were grown in either a hydroponic or a soil-based media for two months before root exudates were collected from respective treatments using a method developed in our laboratory that minimises root damage. We then GC-MS and FTIR Spectroscopy to synergistically analyse fine detail metabolite composition with broad scale metabolic fingerprinting, providing a global profile of exo-metabolomic changes in the collected root exudates.

These spectral data show a marked chemical difference in complex organics in root exudates between plants grown with a hydroponic life history and those grown in soil, despite germination in identical, soil-based conditions. This indicates a degree of environment-dependent control in root exudation and, by assessing the overall impact of hydroponics on exudation chemistry, allows us to gain a better understanding of the fate of exuded metabolites in more natural soil conditions. Such information is critical in discerning the role of specific metabolites in rhizosphere processes.

P.12

ECTOMYCORRHIZA FUNGI INFLUENCE SMALL-SCALE BUT NOT LARGE-SCALE MECHANICAL REINFORCEMENT OF SOIL

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The forest floor is mechanically stabilised by a complex web of tree roots that act as reinforcing elements. Over time, this allows for forests to establish on steep slopes, with tree removal often exacerbating landslide risk. Beyond tree roots, root-associated mycorrhizal fungi may enhance stabilisation processes further, either indirectly by having a positive impact on tree root growth, or by directly affecting the mechanical bonding of soil particles. We explore this latter process by measuring the mechanical behaviour of sterilised soil cores covered in selective meshes that either allowed or excluded fungal in-growth. Cores were buried in forest topsoil, with some treatments periodically twisted to sever hyphal connections to surrounding roots and harvested at 9, 12, 15, and 18 months. Harvested cores were brought back to the laboratory and equilibrated to -5 kPa water potential before mechanical testing. Direct shear tests displaced a top section of the core over a bottom section, creating a defined shear plane that is similar to mechanical conditions in a landslide. There were no differences in shear strength caused by fungal in-growth or hyphal severance at any harvest, but over time the shear strength increased by over 30%. Smaller scale mechanical reinforcement was quantified using a 3 mm spherical indenter, which was pushed in and out of the shear surface to a depth of 1 mm at 5 locations on the newly formed shear surface. Surface hardness, a measure of strength, was 2.4, 2.2 and 1.8 times greater between cores allowing and excluding fungal in-growth for harvests at 9, 12 and 18 months, respectively ($p < 0.01$). Hyphal length measurements of soil within 5 mm of the shear surface found cores excluding fungal in-

growth were almost fungal free. The correlation between hyphal length and mechanical behaviour was poor for the larger-scale direct shear test, but significant for elasticity measured with the smaller-scale spherical indenter.

P.13

PYTHIUM IN CUCUMBER: ALUMINIUM TOXICITY AS A PREDISPOSING FACTOR UNDER ACIDIC CONDITIONS

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Aluminium toxicity is a major constraint to crop production in tropical and sub-tropical acidic soils. However, the effects of Al on soil-borne diseases remain obscure due to diverse pathogenic organisms and lack of appropriate approaches to investigate the interaction of those organisms with Al. Nutrient solution culture provides an appropriate system for the investigation of the interactions. A weak but economically important Oomycete pathogen, *Pythium aphanidermatum*, was used as a model to investigate the relationship between soil-borne diseases and Al-rhizotoxicity in cucumber cv. Beit Alpha. *In vitro* experimentation on agar-based media showed no significant effect of low pH on pathogen growth and pathogenicity. In contrast, there was a significant interaction between pH and *P. aphanidermatum* effects on root growth in nutrient solution culture. In addition to the finding that *P. aphanidermatum* was able to modify media pH, these differences suggested that the conventional approach for investigating acidity and Al effects in pathogenicity might deliver unreliable results. Pathogen establishment in media prior to seedling transfer exhibited detrimental effects across pH levels. Nevertheless, the lack of root length differences between control and three-day-after-transfer inoculated seedlings in nutrient solution culture suggests that low pH *per se* did not predispose roots to infection by *P. aphanidermatum*. Further experimentation with soluble Al at pH 4.5 showed a negative effect on root growth. These findings indicate that Al-toxicity is a stronger predisposing factor to root infection by *P. aphanidermatum* in cucumber than low pH *per se*.

P.14

CHARACTERIZATION OF PLANT GROWTH PROMOTING TRAIT FROM A BARLEY RHIZOSPHERE MICROBIOME USING FUNCTIONAL METAGENOMICS APPROACH

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Functional metagenomics involves isolating DNA from microbial communities to study the functions of encoded proteins. The common work flow of functional metagenome analysis involves the cloning DNA fragments, expressing genes in a surrogate host and screening for activities. Functional metagenomics is recognized to be a useful approach to study the plant growth promotion traits of plant associated microbiomes. In this study barley rhizosphere soil was sampled from a long-term low input phosphate management agricultural site located at Teagasc Oakpark Crop Research Center, in Carlow, Ireland to study plant growth promotion

traits of the barley associated microbiome. A fosmid system was used to clone the metagenome and mineral phosphate solubilization (mps) and organic alkaline phosphatase (alp) traits were screened from 18,000 clones (~666 Mb of DNA). The functional assays and high-performance liquid chromatography analysis recognized gluconic acid production and mps activity in 8 positive clones when screened in an *Escherichia coli* host. The alp activity assay also identified 48 positive hits. The mps clones (with an average insert size of ~37 kb) were analysed by 454 Roche sequencing and annotated. A number of genes/operons with homology to phosphorous uptake and solubilization mechanisms were identified, linking the mps function to the uncultivated microbiome present in barley rhizosphere soil.

P.15

SOIL TILLAGE, CATCH CROPS AND HERBICIDE APPLICATION: A CHALLENGE FOR ARBUSCULAR MYCORRHIZAL FUNGI?

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Arbuscular mycorrhizal fungi are very valuable microbial symbiosis partners in agricultural cropping systems due to their positive impact on plant nutrient uptake, soil and plant health. In a cropping system a multitude of interactions take place. It is well known, that arbuscular mycorrhizal fungi are affected by the tillage system. In contrast, little is known on the impact of cover crops on these beneficial organisms. Moreover, there is an ongoing trend towards conservation tillage systems, which are frequently associated with an increased weed infestation and thus intensifying herbicide usage. We address the question, if the combination of tillage and cover crops or tillage and herbicide usage alter arbuscular mycorrhizal fungi in a field- and field soil-based pot experiments. The experimental field has been under four tillage treatments (conventional, reduced, minimum, no tillage) since 2009. Depending on the tillage system, we found significant differences in the colonization of catch crops and main crops with mycorrhizal fungi. In contrast, arbuscular mycorrhizal root colonization was not affected by the herbicide treatment. Our results support that soil tillage is a major factor for arbuscular mycorrhizal fungi. A better understanding of the interaction of management measures is of great importance for plant growth improvement and plant health.

P.16

DOES SEED SANITIZATION AFFECT THE PLANT RHIZOSPHERE MICROBIOME AND ITS ABILITY TO COMPETE WITH THE HUMAN ASSOCIATED PATHOGEN, *E. COLI* ON SALAD CROPS?

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Cultivation of crops in controlled environmental agricultural systems may limit microbial colonization and reduce diversity of the microbial communities. Practices like seed and growth medium sanitization may further impact microbial communities in the mature plant and the plant's capacity to limit the growth of pathogens through competition. As humans expand their travels to space, understanding plant growth, health, and development in closed environments will be critical to the success of producing a safe, supplemental food source for astronauts. To determine the persistence of a potential human pathogen in plant growth and development, sanitized and unsanitized seeds from, mizuna (*Brassica rapa* var japonica) and red romaine lettuce (*Lactuca sativa* cultivar 'Outredgeous'), were inoculated with *Escherichia coli*, ATCC 11775, germinated under simulated International Space Station (ISS) environmental conditions and harvested every 7 days until maturity. The persistence of *E. coli* in the rhizosphere was determined by plating on selective media, real time PCR and community sequencing of the rhizosphere communities. *E. coli* was detected in the crops' roots and leaves for several weeks post germination. At day 28, plants from sanitized seeds had significantly higher counts of *E. coli* on the roots than those from unsanitized seeds. *E. coli* was also detected on a few uninoculated plants indicating air borne cross contamination among plants in the same growth chamber and suggesting an influence of the natural microbiome on human pathogen survival and persistence in leafy greens. Sequencing analysis revealed variations in composition and diversity between the communities. Understanding the microbial community of the rhizospheric microbiome is only the first step in determining the relationships between plants. Additional studies to include genotypic and phenotypic variations in the plants should be considered to determine if the natural microbes in the rhizosphere may contribute to the health and therefore, safety of the edible plants.

P.17 (will also be presented as poster pitch)

SIDEROPHORE-MEDIATED MICROBIAL INTERACTIONS DETERMINE PATHOGEN GROWTH AND DISEASE INCIDENCE

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Plant resistance to root pathogen is partly driven by rhizosphere microbial species and associated interactions between microbes. Since iron availability of most soils is acknowledged to be limited, we investigated the role of siderophore-mediated interactions, which are critical in iron competition, between resident community and the soil-borne pathogen *Ralstonia solanacearum*. We build up consortia of one to five bacteria and assessed their ability to suppress the soil-borne pathogen *Ralstonia solanacearum* in natural soil. We first confirmed that iron could significantly affect the interactions within resident community and effect of species on pathogen growth, mainly mediated by siderophore. Furthermore, the compatibility of siderophores produced by resident community essentially drove the outcome of infections. Under iron-limited conditions, infection was hampered by resident community producing incompatible siderophores and facilitated when the pathogen could use the community's shared siderophore. These results provide novel mechanistic insights into increased pathogen suppression by probiotic consortia in plant rhizospheres. The siderophore-mediated interactions between bacteria could thus play a key role in engineering functionally reliable microbiome applications.

P.18

HYDROCARBON DEGRADING GENES IN ROOT ENDOPHYTIC COMMUNITIES ON OIL SANDS RECLAMATION COVERS

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The Canadian oil sands industry has expanded rapidly in recent years resulting in a large disturbance footprint. In response to environmental regulations, reclamation strategies aim to return disturbed areas to equivalent land capability prior to mining. However, tailings sands used in oil sands reclamation contain residual hydrocarbons and plants growing in these areas may rely on hydrocarbon-degrading endophytic bacteria to survive. This study assessed the hydrocarbon degradation potential of endophytic communities associated with annual barley (*Hordeum vulgare*) and sweet clover (*Melilotus albus*) growing in an oil sands reclamation area. Three hydrocarbon-degrading genes (CYP 153, *alkB* and *nah*) were (i) quantified in unculturable endophytic bacteria communities using qPCR and (ii) detected in endophytic isolates by endpoint PCR. Our results suggest higher CYP153 gene copy numbers in sweet clover endophytes when compared to barley. Conversely, both plants indicate a similar abundance of 16 rRNA, *alkB* and *nah* genes. Endophytic hydrocarbon degraders in our study also varied based on sampling locations, slope and cover management. In addition, our results suggest that total hydrocarbons, pH, soil carbon and nitrogen play an important role in determining hydrocarbon degrading potential in these communities. The assessment of culturable hydrocarbon degrading bacteria revealed isolates positive for all functional genes analyzed. Out of a total of 316 isolates, 42 isolates were positive for at least one hydrocarbon degrading gene. Most of these isolates indicated the presence of *alkB*, and closely match the database for *Pantoea*, *Pseudomonas* and *Enterobacter* spp. Thus, to improve oil sands reclamation strategies, plant inoculation with select hydrocarbon-degrading endophytic bacteria could be used to increase plant tolerance and hydrocarbon degradation in these areas.

P.19

EVALUATION OF SOIL BACTERIA AS BIOCONTROL AGENTS FOR FIELD PEA ROOT ROT CAUSED BY APHANOMYCES EUTEICHES

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Aphanomyces euteiches is an oomycete pathogen that causes severe rot in the root, cortex and epicotyl of field pea (*Pisum sativum* L) resulting in stunting, yellow and wilting leaves, or plant death. Disease management options are limited, and in Canada none of the traditional and chemical approaches provide complete protection. Therefore, development of biological control agents against this disease may offer an alternative option for growers. The aim of this study was to investigate the potential of soil bacteria to control aphanomyces root rot in field pea. Initial *in vitro* screening of soil bacteria identified 184 isolates (22 from an existing culture collection) that inhibited mycelia and zoospore developmental stages of *A. euteiches*. The mean mycelial growth inhibition potential ranged from 1 mm to 12 mm whereas the mean zoospore germination inhibition ranged from 0 to 100 percent. Based on 16S rDNA gene sequencing, the antagonistic bacterial isolates were placed into 18 different genera. Two sets of growth chamber experiments were conducted using 47 biocontrol bacteria that were most effective and consistently inhibited mycelia and zoospore stages *in vitro*. In the first experiment pea plants were grown in vermiculite, whereas in the second experiment agricultural soil was used. Although most of the tested isolates exhibited biocontrol activity in both growth chamber trials, isolate K-Hf-L9 (*Pseudomonas fluorescens*), PSV1-7 (*Pantoea agglomerans*) and K-Hf-H2 (*Lysobacter capsici*) exhibited the highest biocontrol potential ($\alpha = 0.05$) against aphanomyces root rot in field pea. The results of this study demonstrated the potential promise for the development of bacterial biocontrol agents. Further studies that maximize biocontrol potential and effective utilization are a necessary next step.

P.20

BACTERIAL COMMUNITIES ASSOCIATED WITH BOREAL FOREST HORIZON AND PEAT-MATERIAL IN A OIL SANDS RECLAMATION AREA

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The soil microbiome are intrinsic for establishment of plants and its productivity on land and are associated with seed or propagules and enriched resources such as plant material present in soil. In this study taxonomic composition of soil associated microbiome was compared from a land reclamation area in a oil sands region in Canada to understand the influence of soil covers; the forest organic horizon (i.e., the litter forest horizon, LFH) and peat material commonly used in land reclamation as a top soil cover and its management practices; the fertilization management and woody debris management on the soil associated microbiome. A pyrosequencing based approach was applied to understand the benefits of soil cover and management on the taxonomic composition of bacteria present in these soil. In total, 352,636 sequences were obtained and affiliated to 15 phyla and 30 classes of bacteria. The most dominant phyla and taxonomic groups recognized in these soil were

Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes and Firmicutes (>1% of all sequences). The LFH soil had a higher abundance and diversity of bacteria than peat soil cover and recognized a higher bacterial species richness (OTU's) and diversity (index) in these soil. The benefit of using the woody debris and fertilization management together was also recognized in LFH having higher diversity than peat soil covers. The fertilization by itself increased species richness and diversity in both these soil covers significantly. The Proteobacteria organisms with Beta and Delta class of Proteobacteria and Bacteroidetes were more abundant in LFH soil covers, whereas Acidobacteria and Firmicutes were recognized to be higher in peat covers and under these management or practices. The results of this study therefore suggest that soil material and management have influence on bacterial communities which should be assessed as to their potential to assist plant establishment at these sites.

P.21

PHOSPHOBACTERIA AND TRIPLE SUPERPHOSPHATE DOWNREGULATE PHOSPHATE TRANSPORTER AND SUPEROXIDE DISMUTASE GENE EXPRESSION IN LOLIUM PERENNE

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Phosphobacteria favors plant performance in acidic soils by increasing phosphorus (P) availability. However, it is not well known how phosphobacteria affects plant gene expression. This study aimed to evaluate the ability of a phosphobacteria consortium to regulate gene expression of P-transporters and superoxide dismutase isoforms in ryegrass grown in two Andisols from southern Chile. A greenhouse assay was established in two Andisols, Barros Arana and Gorbea, selected for their contrasting P availability. Ryegrass plants grown in each soil were subjected to four treatments: P-B- (control); ii) P-B+ iii) P+B- and iv) P+B+, where P-: P unfertilized plants; P+: plants fertilized with triple superphosphate; B-: Uninoculated plants and B+: plants inoculated with a consortium formulated five phosphobacteria strains: *Klebsiella* sp.RC3, *Stenotrophomonas* sp.RC5, *Klebsiella* sp.RCJ4, *Serratia* sp.RCJ6 and *Enterobacter* sp.RJAL6. After six weeks, relative expression of genes encoding for three P-transporter (*LpPHT1;1*, *LpPHT1;4* and *LpPHT1;6*) and superoxide dismutase isoforms (*Mn-SOD*; *Fe-SOD* and *Cu/Zn-SOD*) was evaluated by real-time PCR. Our results revealed that *LpPHT1;1* and *LpPHT1;4* expression was downregulated in shoot of inoculated and fertilized ryegrass grown in both soils. Similarly, superoxide dismutase isoforms expression was downregulated in roots and shoots of plants grown in Barros Arana soil by effect of P-fertilization, which was potentiated for phosphobacteria inoculation. The P content in ryegrass tissues was increased by P fertilization and bacterial inoculation. Therefore, phosphobacteria consortium inoculated along with fertilizer have the ability to induce changes in the ryegrass genetic expression patterns, which is accompanied by greater biomass, P content and reduced cellular damage. Consequently, the extended

application of phosphobacteria as biological fertilizer in P-poor soils is an attractive and potentially useful complement to P fertilizers.

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P.22

APPLE CULTIVAR SHAPE THE RHIZOSPHERE SOIL MICROBIOTA OF DIFFERENT SCION/ROOTSTOCK COMBINATIONS

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In the complex ecosystem of apple orchard, grafting is a common production mode. In general, scion can affect the root phenotypes of grafted apple by the sugar and hormone contents. Roots can acquire water and nutrients from soil, and are the main niche of soil microorganisms, but little is known whether different scions influence the rhizosphere bacteria recruited from soil. We used 16S rRNA high-throughput sequencing to characterize the rhizosphere bacteria of two apple varieties ('Golden delicious' and 'Fuji') grafted on seven different rootstocks, respectively, and all of the scion/rootstock combinations were cultivated in the same orchard. The results indicated that rootstocks influence the structure of rhizosphere bacterial communities, but microbial communities were more similar when grafted on the same scion. The relative abundance of Gemmatimonadates, Chloroflexi and Nitrospirae increased when 'Fuji' used as the scion, while Actinobacteria and Firmicutes were the opposite. The root of seven rootstocks grafted with 'Fuji' elevated soluble sugars content. In especial, the content of sucrose was more influenced by scion varieties. Our finding suggested that apple scions can regulate the content of soluble sugar in root, especially sucrose, to affect the content of organic carbon in rhizosphere soil, as to regulate the composition and structure of rhizosphere bacterial community of different scion/rootstock combinations.

P.23

DIFFERENT METABOLIC POTENTIALS BETWEEN FIRMICUTES AND OTHER PHYLA IN A SOIL AMENDED WITH MANURE

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Recently there are renewed interests in utilizing manure because of law-requested land reclamation and of a manure utilization motivation scheme in China. Land reclamation converts land surface materials to a fertile

soils using manure amendment, which shifts a low-carbon adapted microbial community to a high-carbon adapted community in a high resolution. Purpose of the present study is to investigate the response of microbial community to manure amendment and the relationship between taxonomic composition and metabolic potentials. We used shotgun metagenomic approaches to profile soil metabolic potentials and correlate them with taxonomies. The results revealed that the abundance of Firmicutes decreased after manure amendment. Correspondingly, the abundance of KEGG pathways associated with cell-wall biosynthesis, membrane transports and carbohydrate active enzymes (CAZymes) also decreased. On the contrary, the abundance of other phyla, particularly Betaproteobacteria, Bacteroidetes and Verrucomicrobia, increased after manure amendment. Correspondingly, the abundance of KEGG pathways associated with lipopolysaccharide biosynthesis, bacterial motility and carbon metabolisms that include CAZymes also increased. These data demonstrated that the phylum Firmicutes were enriched more with genes associated with cell-wall biosynthesis and membrane transports while other phyla were enriched more with genes associated with lipopolysaccharide biosynthesis and carbohydrate metabolism, supporting our hypothesis that the phylum Firmicutes has a lower potential of utilizing manure-derived carbohydrates than other phyla. Thus, manure added supported Betaproteobacteria, Bacteroidetes and Verrucomicrobia, but suppressed Firmicutes. Our results add an additional information on the ecological classification of soil microorganisms and support the viewpoint of ecological coherence of high bacterial taxonomic ranks.

P.24 (will also be presented as poster pitch)

SELECTED BACTERIAL ISOLATES FROM LENTIL FIELD SHOWED ANTAGONISM ACTIVITY AGAINST APHANOMYCES EUTEICHES

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Aphanomyces root rot is becoming one of the major threats to lentil (*Lens culinaris* Medik.) production in Canada. No known seed treatments and/or conventional control measures are effective against this pathogen. Thus, the discovery and validation of alternative *Aphanomyces euteiches* control strategies, such as bacteria antagonistic to *A. euteiches*, is critical for successful lentil production. In this study, rhizosphere and root samples of both diseased and healthy lentil plants were collected across southern Saskatchewan, and over 10,000 bacteria isolates were cultured from these samples in lab. Among these bacterial isolates, 16 of them which showed antagonism activity based on previous tests were screened for the greenhouse experiment. Under greenhouse condition, 12 of these 16 bacterial isolates showed strong antagonism activity against *A. euteiches* while other four showed moderate antagonism activity, indicating potential capacity for using antagonistic bacteria to control root rot disease caused by *A. euteiches* in lentil field.

P.25

COVER CROPS AS DRIVERS FOR *SCLEROTINIA SCLEROTIORUM* IN SOYBEAN PRODUCTION

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Cover crops are becoming increasingly important in sustainable agriculture. They are characterized by their beneficial effect on soil health, but many of them are suspected being hosts for plant pathogens like *Sclerotinia sclerotiorum*. This soil borne fungus is able to survive as sclerotia for several years and can cause enormous yield losses in many crop plants, among others in soybeans (*Glycine max*). In a three-year field experiment 15 cover crops (and bare fallow as control) were tested for their impact on *Sclerotinia sclerotiorum* development and the subsequent crop soybean. Sclerotia were produced under laboratory conditions, filled in mesh tubes and incorporated into the soil to a depth of 3 cm after cover crop seeding in July. In April of the following year the sclerotia were excavated, counted and prepared for the re-inoculation in soybean in May. Regular examinations were done for apothecia development and plant infections. Only a few cover crops, such as mustard (*Sinapis alba*), grass pea (*Lathyrus sativus*), phacelia (*Phacelia tanacetifolia*) and corncockle (*Agrostemma githago*), were infected by *Sclerotinia sclerotiorum*. In the subsequent year the impact of the cover crop and *Sclerotinia sclerotiorum* on soybean yield depends on cover crop development in autumn and plant available water before and during soybean flowering. However, in comparison to bare fallow most cover crops led to a better or at least same soybean performance.

P.26

COLONIZATION PATTERNS OF ROOT ENDOPHYTIC *SERENDIPITA* SPP. IN A PLANT-PATHOGEN SYSTEM

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The application of biocontrol agents (BCAs) as an alternative to chemical pesticides and synthetic fertilizers in agricultural systems is gaining interest, as pathogen resistance and extreme weather conditions challenge food production all over the world. Still, little is known about fungal endophytes and their putative role as BCAs with regards to soilborne pathogens. The root endophytic fungus *Serendipita indica* (syn. *Piriformospora indica*, Basidiomycota) is able to establish beneficial interactions with a high number of plants, resulting in enhanced plant growth and increased resistance against pathogens. Its lesser-known close relative *S. williamsii* (syn. *P. williamsii*) was isolated from European sites and shows similar characteristics. For successful biological control, a detailed knowledge on the mechanisms involved is essential. This also includes knowledge on the colonization patterns in the rhizosphere and in the different root tissues.

The aim of this study was to shed light on the colonization process of *S. indica* and *S. williamsii* in tomato roots (var. “Kremser Perle”) in the absence or presence of the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). Tomato seedlings were inoculated with *gfp* (green fluorescent protein) expressing *Serendipita* spp. and *tdtom* (red fluorescent fluorophore) expressing *Fol* and roots were observed with a confocal laser scanning microscope at different time intervals. All fungi could be visualized at the rhizoplane and inside the tomato root. First observations revealed co-occurrence of the endophytic *Serendipita* spp. and the pathogenic *Fol*. Their colonization patterns and local interactions are currently being further investigated.

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P.27

DIVERSITY AND GROWTH PROMOTING POTENTIAL OF BACTERIAL ENDOPHYTES ASSOCIATED WITH CROPS

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Bacterial endophytes can enhance crop yield by synthesizing plant growth regulators, facilitating nutrient uptake from the soil and/or inhibiting phytopathogen activity. Recent studies indicate that the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is also an important mechanism involved when improving crop productivity. In this study we assessed the diversity and plant growth promoting potential of bacteria associated with the root interior of canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown on agricultural soils in Saskatchewan. Production of ACC deaminase by bacterial endophytes was investigated as a possible mechanism involved in plant growth promotion in agricultural crops. A total of 298 endophytic bacteria isolated from the four crops using culture dependent methods included the genera *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium* and *Stenotrophomonas*. Of these, 40 isolates that promoted highest seed germination rates were tested for root elongation and ACC deaminase production *in vitro*. Isolates *Agrococcus carbonis*, strain WCB1-23; *Leifsonia xyli*, strain WM17; *Paenibacillus taohuashanense*, strain WCB2-2; *Pantoea vagans*, strain CS1-1; *Pseudomonas* sp., strain PM1-1; *Rhodococcus cerastrii*, strain WCB1-10; *Stenotrophomonas rhizophila*, strain WCB2-14 and *Xanthomonas fuscans*, strain WK1-6 increased the root length in canola whereas, only isolate *Stenotrophomonas maltophilia*, strain CM3-1 promoted root growth in wheat. In our study, production of ACC deaminase was detected in 16 bacterial endophytes, of which six strains increased root elongation, thus indicating that ACC deaminase activity is not the key mechanism involved in growth promotion, by these endophytes. Overall, this study indicates that crops are colonized by a diverse group of bacterial endophytes with potential to increase plant growth in agricultural ecosystems.

P.28

IDENTIFICATION OF A BIOCONTROL FOR AGROBACTERIUM VITIS, THE CAUSAL AGENT OF GRAPEVINE CROWN GALL

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Crown gall of grapevines, caused by the soil bacterium *Agrobacterium vitis* (*A. vitis*), is an economically important disease in grape-growing regions with a continental climate, particularly where winter freezing occurs. Symptoms include galls that significantly reduce plant vigor leading to vine death. The disease is present worldwide, and many vineyards in British Columbia are severely infected. The pathogen survives systemically in vines and is present in vineyard soil, from where it enters the vine through wounds in plant roots. No chemical control is available to date. Biocontrol using an avirulent *Agrobacterium radiobacter* strain K84 strain has been successful in preventing crown gall in fruit trees, but no biocontrol is currently commercially available for grapevine crown gall. The aim of this study was to identify a biocontrol strain that is present in British Columbia soils and thereby adapted to local environmental conditions. Soil was sampled from seven vineyards in the Okanagan valley of British Columbia. Bacteria were isolated from rhizosphere soil and from vine roots and plated onto Roy and Sasser (semi-selective for *A. vitis*) or tryptic-soy agar (general) plates. A total of 472 bacteria were isolated and a bacterial collection prepared. The collection was screened for growth inhibition of *A. vitis* on Luria Bertani medium plates. Sixteen, 14 and 4 isolates showed weak, medium and strong inhibition of *A. vitis*, respectively. The isolates are undergoing further testing on plates and will be inoculated into tomato plants to test for inhibition of gall formation *in-planta*.

P.29

THE IMPACT OF LONG-TERM FERTILIZATION MANAGEMENT ON RHIZODEPOSITION AND ITS ROLE ON THE RHIZOSPHERE MICROBIAL COMMUNITIES AND HEALTH STATUS OF LETTUCE

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Long-term field trials offer excellent options to study the impact of agricultural management on soil microbial communities and soil biota as related to crop performance. For this study, lettuce was grown as a model plant on soils from two long-term field experiments comparing organic versus mineral fertilization management.

Growth and performance of lettuce plants were strongly influenced by the history of soil management. Unexpectedly, on a long-term organic farming soil with biodynamic management, root infection by the pathogen *Olpidium brassicacearum* was reflected by reduced plant biomass production and stunted root growth. This was associated with increased expression of pathogen-related genes in lettuce leaves (RbohD; PDF1.2, WYRK25, OPT3) and increased root accumulation and exudation of stress metabolites (proline). At the bacterial community level, the abundance of *Gammaproteobacteria* (GPB), representing the dominant bacterial class in the lettuce rhizosphere (approx.27-40%), was drastically reduced to 8.6 % in the rhizosphere of *Olpidium*-affected plants. This was associated also with a reduced rhizosphere abundance of the GPB-genus *Pseudomonas* containing strains frequently associated with plant growth promotion and pathogen suppression. Contrary to the known substrate preferences of GPBs, the sugar/amino acid ratio in the root exudates of the pathogen-affected plants declined by more than 90 %, while non-affected plants showed higher benzoic acid exudation with antibiotic properties.

Taken together the results indicate that alterations in root exudation on the non-affected soils directly promoted the soil pathogen-suppressiveness by increased release of antibiotic compounds and indirectly by providing appropriate C/N sources supporting the proliferation of potential pathogen- suppressive bacterial taxa in the rhizosphere.

P.30

GENETIC DETERMINANTS NEEDED FOR RHIZOBIUM LEGUMINOSARUM TO COLONIZE GERMINATING PEA SEEDS

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Soil is one of the most complex environments and the rhizosphere is a substantial component. The consortium of rhizobium and rhizosphere has been studied extensively to understand the importance of the rhizosphere in establishing the specific plant-microbe association. A seed goes through different stages of germination before reaching to a full grown root system. The spermosphere is an early and short stage of seed germination. The spermosphere can have an effect on building a connection between a specific microbial community and its host plant but this environment remains relatively under studied.

The purpose of this study was to identify genes in *Rhizobium leguminosarum* that impart a fitness benefit to colonization of the spermosphere environment of pea seeds. A high throughput genetic screening technique, Insertion Sequencing (INSeq) has been applied to identify genes which aid the bacteria to adapt in this specific spermosphere environment. From this study, 12 genes were found to uniquely have a fitness impact during colonization of the emerging radicle of pea seeds. Validation of the INSeq data has been done by selecting one of the genes (RL3994) and creating an insertional mutation. RL3994 is a putative acid tolerant protein which is the second gene of a two gene operon. We found reduction in growth of RL3994 mutant compared to wild-type (WT) in lower pH of 6.2 and 5.8. The mutant is also found to be a poor competitor in pea nodulation competition experiment.

Keywords: Spermosphere, Radicle, Rhizobium, INSeq, Mutagenesis

P.31

SOIL MICROBIOMES ACHIEVE ALTERNATE STABLE-STATES AFTER TWENTY YEARS OF CROP ROTATION

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Agricultural soil microbiomes are shaped by bottom-up and top-down factors, including soil physico-chemical characteristics, crop composition, management practices and climate factors. Practices such as crop rotation are thought to increase microbiome diversity and thus enhance the provision of microbiome-mediated ecosystem goods and services. We still do not know however, how long it takes for a new crop rotation practice to alter these microbiomes, whether the new assemblage is stable, or if that altered stable state is inherently beneficial to crop production. To investigate these questions we sampled monoculture and rotation crop treatments at a long-term Agriculture and Agri-Food Canada crop rotation trial (Woodslee, Ontario; established 2001) in the spring and fall for 3 cropping seasons. Continuous corn, soybean, and wheat monoculture, corn-soybean-wheat rotations (each phase present every year), and 2 perennial grass treatments (n=4 all treatments/phases) were sampled to a depth of 0-15cm. The relative impact of impact of rotation history, current crop phase, current crop stage, and climate on bacterial and fungal population dynamics was evaluated using meta-barcode sequencing. Our findings show that after approximately 20 years of rotation, the legacy effects of crop rotation on soil microbiomes supersedes those of the current crop in the system. Microbiomes were fundamentally altered, achieving alternative stable-states that were also resilient to climate pressures. Understanding the relative impacts of legacy versus immediate biotic/abiotic impacts on soil microbiomes provides a starting point from which to incorporate microbiome parameters into precision farming initiatives.

P.32

BACTERIAL COMMUNITY STRUCTURES OF RHIZOSPHERE SOIL AND ROOT OF LATE MATURING CITRUS CULTIVATED IN P. E. HOUSE

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The cultivation of citrus varieties is increasing with the use of houses. In order to produce delicious bananas, it is necessary to manage the amount of soil and moisture in the house until harvest time. Nutrient sources supplied to the soil for crop cultivation are decomposed and absorbed by microorganisms. The characteristics of soils, kinds of plant materials, application capacity and soil moisture condition affect the community structure and diversity of soil microorganisms. The community structure and diversity of bacteria distributed in soil and roots of the soil were evaluated. In May, soil and roots were collected from non - volcanic ash soil (3 sites) and volcanic ash soil (4 sites). The soil of non - volcanic ash was dry and the soil of volcanic ash was organic soil and irrigation. Bacterial populations, species richness, diversity index and bacterial population distribution were investigated by sequencing of the next generation after soil extraction. Rarefaction curves showing similarity of 97% sequence showed more OTUs in rhizosphere soil than root area. The average OTUs of bacterial populations were similar to those of non - volcanic ash, 4,868, 1,995.7, 5,012.5, and 1,961.5, respectively. The species richness estimates Ace and Chao1 of the bacteria were similar regardless of the soil, and the rhizosphere

soil was higher than the roots. Shannon diversity index was higher than that of roots. The dominant bacterial groups were Proteobacteria, Acidobacteria, Actinobacteria and Chloroflexi.

P.33

SOIL BINDING POLYSACCHARIDES AND THE FORMATION OF THE RHIZOSHEATH

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Rhizosheaths are proposed to have an important role in plant-soil interactions. They have been implicated in drought tolerance and has different water holding capacity to bulk soil. They are formed by the entanglement of soil particles by root hairs and adhesive root exudates. Not much is known about the adhesive root exudates released from roots. Some cell wall-like polysaccharides, such as xyloglucan, have shown soil binding properties. Using a novel high-throughput method high molecular weight compounds (HMWC) released from the roots of maize and wheat have shown to effectively bind soil with wheat binding more effectively than maize. The root exudates have been characterised and have been shown to contain xyloglucan, heteroxylan, arabinogalactan protein (AGP) and extensin epitopes using immunohistochemical techniques. In maize root exudate no extensin epitope is found and the other epitopes are found in lower amounts. Removal of polysaccharides decrease the soil binding capacity of the HMWC in both maize and wheat. These epitopes have also been found to be released along the whole root body. This suggests that polysaccharides are released as root exudates and are an adhesive factor in the formation and stabilisation of the rhizosheath.

P.34

AMELIORATIVE EFFECTS OF BORON ON ALUMINUM-INDUCED INHIBITION OF ROOT GROWTH IN TRIFOLIATE ORANGE (PONCIRUS TRIFOLIATE (L.) RAF.) ROOTSTOCK

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Acid soils comprise up to 40% of the world's potentially arable lands. Aluminum toxicity is the main constraint of root growth in arable acidic soil. Boron is a necessary nutrient for normal growth and development of plants. Although boron was used to ameliorate aluminum stress, the exact mechanisms of boron alleviates aluminum toxicity is sparse especially in the trifoliolate orange which is important rootstock in China. In this work, trifoliolate orange seedlings were irrigated in a nutrient solution containing two boron levels and two aluminum levels. The main results showed that aluminum toxicity severely hampered the root elongation and the plant growth-related parameters, caused oxidative stress in terms of H₂O₂ and O₂⁻, membrane damage, and severe root injury under boron deficiency. Interestingly, under aluminum exposure, boron supply improved superoxide dismutase activity while reducing peroxidase, catalase and polyphenol oxidase activities. Likewise, the contents of H₂O₂, lipid peroxidation, protein and proline in roots were markedly decreased by boron application under aluminum exposure. Besides, boron supply reduced the alkali-soluble pectin under aluminum toxicity. Fourier transform infrared spectroscopy and ¹³C nuclear magnetic resonance spectra revealed the decrease of carboxyl groups and cellulose by boron application during aluminum exposure. Furthermore, boron supply decreased the

aluminum uptake, cell wall thickness and callose formation. These results imply that boron could reduce aluminum-induced negative effects on plant growth by regulating antioxidant enzyme activities in the roots. Meanwhile, boron could mitigate aluminum phytotoxicity by shielding potential aluminum binding sites and by reducing aluminum induced alterations in the cell wall cellulose and pectin components, which ultimately results in reduced root injury and improved root growth. The results provide useful insights into the mechanism of boron -induced aluminum toxicity reduction, especially for the application of boron fertilizer in acidic red soil, which is helpful to the agricultural development of citrus.

P.35

UNRAVELLING THE ROLE OF BORON IN PROMOTING RHIZOSPHERE ALKALINIZATION IN THE ROOT TRANSITION ZONE

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Boron (B) is an essential micronutrient that functions mainly in the cell wall. Our recent research suggested a novel role of B in promoting root growth by maintaining rhizosphere alkalization in the transition zone of root apex via regulation of plasma membrane H⁺-ATPase (PM-H⁺-ATPase). Alkalinization of root apices (0-3 mm) and acidification of distal zones (3-10 mm) of pea plants (*Pisum sativum*) was observed by measuring the rhizosphere pH of ten sequential 1-mm-long sections isolated from root apices, using the Ultra-Micro pH meter. Root apical alkalization was also reported by the BCECF-dextran fluorescent imaging and further confined to the root transition zone (1-2 mm) by measuring pH values along the root axis using H⁺-selective microelectrodes adopting noninvasive microtest technology (NMT). Rhizosphere alkalization in the root transition zone was found in B-sufficient plants but not in B-deficient plants in both BCECF-dextran and NMT tests, whereas root apical acidification was indicated in B-deficient plants by bromocresol purple in agar. These results are explained by the role of B in promoting H⁺ efflux in the root meristem and elongation zone while promoting H⁺ influx in the transition zone. Plasma membrane hyperpolarization detected by the MIFE technology matched the relatively lower net H⁺ influx in the cells of root transition zone in B-deficient plants. Pharmacological tests with fusicoccin (FC) and vanadate (VA) suggested that rhizosphere alkalization was attributed to the role of B in stimulation of PM-H⁺-ATPase in the root transition zone (inhibited by VA but stimulated by FC). Importantly, the beneficial role of B supply on root growth disappeared in buffered solutions or in the presence of VA, which inhibited PM-H⁺-ATPase. In summary, this study suggests a novel role of B in promoting root growth by maintaining rhizosphere alkalization in the root apex transition zone via regulation of the PM-H⁺-ATPase activity.

P.36

CHARACTERIZATION OF PLANT GROWTH PROMOTING BACTERIA FROM HALOPHYTES RHIZOSPHERE AND THEIR EFFECT ON MAIZE GROWTH

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The microbial diversity in the rhizosphere can provide information regarding various mechanisms enabling plants to survive under extreme conditions of abiotic stress. Rhizobacteria play an important role in plant health and soil fertility. The main objective of the present work was to study plant growth promoting potential of bacterial strains from the rhizosphere, rhizoplane and root endosphere of halophytes (*Salsola stocksii* and *Atriplex amnicola*). On the basis of salt tolerance, forty-one bacterial isolates were selected for molecular characterization and plant growth promoting traits. Phylogenetic analysis showed that bacterial strains belonging to *Bacillus*, *Halobacillus*, *Halomonas*, *Enterobacter* and *Pseudomonas* were dominant in the rhizosphere of halophytes. Out of 41, 35 isolates showed P-solubilization activity, 31 strains showed production of indole acetic acid, 19 bacterial isolates showed nitrogen fixing ability, 7 isolates showed siderophore production and 5 strains showed HCN production. To study the plant growth promoting effect on maize, five bacterial strains; *Bacillus safensis* HL1HP11 and *Bacillus pumilus* HL3RS14, *Kocuria rosea* HL1RP8, *Enterobacter aerogenes* AT1HP4 and *Aeromonas veronii* AT1RP10 were used as inoculants; in the form of seed coat and enriched soil-based phosphate biofertilizers. All bacterial strains positively affected the maize growth as compared to non-inoculated control plants. Plants inoculated with *Bacillus* HL3RS14 based soil biofertilizers showed maximum increase in dry weights of root (64-134%) and shoot (45-119%) as compared to control (soil + rock phosphate, no inoculum). PGPR inoculations under salinity stress conditions showed high concentrations of osmolytes such as proline and glycine betaine. These results indicated that under saline affected lands, halophilic bacterial strains having plant beneficial traits are more promising candidates as biofertilizers when used with carrier materials.

P.37

ASSESSMENT OF THE LEGACY P BY BRACHIARIA AND MAIZE IN TROPICAL AND TEMPERATE SOILS

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The surplus of P accumulated in croplands due to the excessive and intensive application of P fertilizers is commonly termed 'legacy P', and it may represent a substantial secondary P resource to postpone the worldwide crisis of rock phosphate demand. According to some references, legacy P is able to support globally crop P demands for approximately 9–22 years, with more expressive long-term support in Europe and Oceania and less support in America and Africa. This work aimed to estimate, by cropping and laboratory procedures,

how much of the legacy P accumulated in the soil over the long time of fertiliser usage is plant useful/profitable for crop production. This short term estimative was based on ten distinct soils from Brazil (tropical region) and six distinct soils from UK (temperate region), all representatives of the main croplands of each country/regions. The study was carried at Bangor University, Bangor-UK. Soils were sampled from 0-20 cm layer from ten locations in seven States of Brazil, being mostly Ferralsols, and from Bangor and five ADAS research centers in UK (Gleadthorpe, Terrington, Boxworth, Drayton and Rosemaund). All those soils were submitted to cultivation of brachiaria and maize for six consecutive cycles of 28-32 days in pots of 1 kg. Soil P depletion was estimated by Hedley chemical fractionation procedure before and after greenhouse cultivation. Crop response in dry matter yield and P uptake was directly influenced by the amount of labile P present in the soil, with much better results from UK soils compared to Brazilian soils. Rosemaund and Bangor were the most fertile and productive soils evaluated here. Both brachiaria and maize were able to deplete labile inorganic P and mobilise P from mod- and non-labile fractions. Also, brachiaria was more effective to potentially mobilise P_{Residual} to more labile fractions than maize.

P.38

SOIL MICROBIAL BIOMASS PHOSPHORUS RESPONSE TO PHOSPHORUS APPLICATION AND ROOT CARBON DEPOSITION

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Rhizosphere and bulk soils are distinct environments for soil microbes. In the rhizosphere soil, microbes receive substantial carbon released from plant roots. While in the bulk soil, microbes are affected more severely by fertilizer addition. Understanding the responses of microbial biomass to carbon deposition and inorganic fertilizer addition is key to enhance the plant growth and yield.

In the present study, we conducted two field experiments with different P application rates to assess soil microbial biomass phosphorus in the rhizosphere and bulk soil. Results showed that soil microbial biomass phosphorus in the rhizosphere was significantly greater than bulk soil. Although plant available phosphorus (Olsen-P) had a positive relationship with microbial biomass phosphorus, there was no significant difference in the rhizosphere compared with the bulk. Olsen phosphorus was not a main factor for the change of microbial biomass. Consistent increases of dissolved organic carbon at maturity and dissolved organic carbon depletion in the rhizosphere at flowering stage indicated that the increase of rhizosphere microbial biomass phosphorus was attributed to root carbon deposition. Microbial biomass phosphorus responded to the ratio of soil organic carbon and Olsen phosphorus indicating that the distinct critical value in the rhizosphere (1688) and bulk soil (695) were different.

Taken together, our results suggest that root carbon deposition plays a key role in microbial biomass turnover in the rhizosphere soil. Soil organic carbon and Olsen phosphorus determined microbial biomass in the bulk soil. Understanding the regulation of this in the soil environment may further improve the management of microbial biomass phosphorus which could benefit phosphorus utilization by crops.

P.39

ACTINOBACTERIA DIVERSITY AND CHITINASE C GENE ABUNDANCE IN CANADIAN ORCHARD SOILS UNDER DIFFERENT LAND MANAGEMENT

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Bacterial chitinases are considered important factors in suppressive soils as they can degrade the cell wall of pathogenic fungi and the outer layer of plant parasitic nematodes. Among the soil microbial chitinolytic communities, Actinobacteria members have been documented as the most important taxa. Land management practices and agricultural use can affect the natural microbial communities present in the soil. The aim of this work was to study the effect of soil amendments (compost and mulch), fumigation, and land use history on the actinobacterial diversity and the soil chitinases genes abundance. Soil DNA was extracted, amplified for RNA16s and sequenced to study the actinobacterial biodiversity. *chiC* gene of Streptomyces was used as an indicator for bacterial chitinases and its abundance was determined by digital droplet PCR. Results showed a detrimental effect of the land agricultural use on the abundance of chitinase *chiC* gene as compare with non-farmed soils. The abundance of bacterial chitinases genes correlated positively with higher soil organic matter and new orchards, and negatively with pH and high potassium content. The organic amendments did not improve significantly the chitinolytic communities. A total of 78 actinobacterial genera were determined, 30 of them unidentified. Agricultural management impacted the biodiversity of orchards soils modifying the actinobacterial composition pattern. Overall our study suggested that actinobacterial biodiversity patterns were substantially influenced by cherry cultivation and the different geographical location of the orchards.

P.40

DO MULTI-SPECIES COVER CROP MIXTURES INCREASE NITROGEN ON ABOVEGROUND AND ROOT IN ORGANIC CROPPING SYSTEM?

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Nitrogen management is particularly challenging for organic producers since they are not allowed to use mineral sources of nitrogen. Therefore, they rely heavily on legume cover crops for nitrogen input. In Quebec, there is a recent interest for multi-species cover crop mixtures. However, there is a lack of information about the efficiency and the profitability of these complex cover crop mixtures (over six species). Moreover, few studies take nitrogen content of root cover crops into account. The main objective of this project was to evaluate the effect of multi-species cover crop mixtures on nitrogen uptake and yield of a subsequent corn crop without synthetic inputs under Quebec's climatic conditions. The specific objective regarding the study of cover crop roots was to determine the effect of cover crop mixtures on nitrogen in aboveground and root biomass. A field experiment was conducted in 2017 and 2018 at Laval University Experimental Farm at Saint-Augustin-de-Desmaures (Quebec). Five cover crop treatments (no cover crop and

1-2-6-12 cover crop species) were fall-seeding in August following spring barley harvest. Cover crop aboveground and root biomass were measured in fall prior to frost-killing. Root biomass was taken at two soil depths (0-15 cm and 15-30 cm) in the same 0.25 m² quadrats as aboveground biomass. Total nitrogen and carbon content of aboveground and root biomass were also measured. Using a mixture of cover crop species did not result in higher aboveground and root biomass than a pure stand of field peas. Cover crop mixtures neither provided a higher nitrogen content in their above ground and root biomass. Our project also assessed other ecosystem services provided by cover crop mixtures such as soil mineral nitrogen supply and corn yield. Further study should consider functional traits of roots for predicting nitrogen availability for subsequent crops from cover crops mixtures.

P.41 (will also be presented as poster pitch)

EFFECTS OF X-RAY COMPUTED TOMOGRAPHY ON ROOT TRANSCRIPTOME AND RHIZOSPHERE MICROBIOTA

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While plant responses to multiple members of the rhizosphere microbiome have been characterized, and various interactions and diverse chemical dialogues that occur in the rhizosphere have been unravelled, our understanding of the spatial distribution of rhizosphere processes is limited. To gain a spatial insight into the genes and functions playing out in rhizosphere processes, we aim to combine non-invasive imaging with spatial sampling and analysis. This approach starts by X-ray computed tomography (CT) and the information from the CT scans is subsequently used to dissect roots and isolate rhizospheres in a spatial context. CT is a powerful technique, since it can not only be used to observe and quantify root systems, but also to assess soil-plant relations. Nevertheless, a critical aspect of CT studies is: Does the used dosage of radiation influence living organisms? This might occur directly by e.g. evoking oxidative stress, or indirectly via changed levels in plant and microorganism available nutrients. To target this question, the impact of CT on maize gene expression profiles, enzyme activity levels, rhizosphere microbiome composition and soil enzyme activities will be investigated. A controlled soil column experiment with 3-wk-old maize seedlings was implemented and great care was taken to disturb the plant-soil system as little as possible during the application of CT. In order to obtain a comprehensive view on the biotic responses to the X-ray dosage, our analysis focuses on early (30 minutes after CT) and late (24 hours) responses of maize and microbiota. We will present maize RNA sequencing data to reveal how CT affects the root transcriptome and rhizosphere soil enzyme activity profiles to evaluate the impact of the X-rays on microbial activities. This research was conducted within the research program “Rhizosphere Spatiotemporal Organisation – a Key to Rhizosphere Functions” of the German Science Foundation.

P.42

IS pH THE KEY REASON WHY SOME LUPINUS SPECIES ARE SENSITIVE TO CALCAREOUS SOIL?

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The calcifuge syndrome is often explained in terms of soil pH or pH-buffering capacity, and this explanation is satisfactory in many cases. However, for species that produce carboxylate-exuding (cluster) roots, this classic explanation makes little sense, because these species would have the capacity to strongly acidify their rhizosphere, and thus mobilise nutrients that are poorly available at a high pH. Therefore, we hypothesised that the reason why some *Lupinus* species are insensitive to calcareous soils is that they cope better with a high calcium (Ca) supply. We further hypothesized that they accumulate phosphorus (P) in mesophyll cells, and Ca in epidermal cells, thus avoiding the precipitation of calcium phosphates, which would render both Ca and P unavailable. We tested this hypothesis by growing different *Lupinus* species in nutrient solution or soil and measured biomass, leaf and root nutrient concentrations (including P and Ca), gas exchange, chlorophyll fluorescence, and P- and Ca-uptake rates. We also assessed cluster-root formation and activity under all treatments (pH, different calcium chloride and potassium bicarbonate concentrations, and combinations thereof). Finally, we determined the cell types in which Ca and P accumulated, using elemental X-ray microanalyses of leaf samples.

Our studies confirmed that Ca partly explains the calcifuge habit of some *Lupinus* species, because Ca and P precipitated in some species and caused P-deficiency symptoms. However, high pH (resulting from addition of either KOH or HCO₃⁻) inhibited root growth, and subsequently uptake of some nutrients, and shoot growth of *Lupinus* species. This explains why some *Lupinus* species are sensitive to calcareous soils, and the strong buffering capacity of HCO₃⁻ is the key factor determining if *Lupinus* species can function in calcareous soils or not.

P.43

EFFECTS OF BIOCHAR ON SPATIAL AND TEMPORAL LOCALIZATION OF ENZYME ACTIVITIES IN MAIZE RHIZOSPHERE

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Enzymes secreted by microorganisms and roots catalyze nutrient release from soil organic matter. Application of biochar affects enzyme activity, but enzyme-biochar interactions are still unclear especially in relation to enzymatic hotspots.

Soil zymography was used to visualize the spatio-temporal distribution of activities of three enzymes (β -glucosidase, acid phosphatase and leucine-aminopeptidase) in maize rhizosphere. A split-root system was adopted to test response of root and rhizosphere microbial activity to biochar amendment and fertilization. Rhizoboxes were filled half-half with soil treated either with biochar, nitrogen fertilizer or untreated control soil.

Biochar decreased the hotspot area of β -glucosidase by 57~96% and acid phosphatase by 59~99% compared to the control compartment, and hotspot area of β -glucosidase was 61~75% lower in biochar than in nitrogen fertilized compartment. Hotspot area of leucine-aminopeptidase was not affected by biochar. Over a period of 30 days, hotspot area of β -glucosidase in all biochar compartments increased, while that of acid phosphatase and leucine-aminopeptidase in biochar compartments was highest at the middle growth stage.

Only maize in control compartment next to biochar compartments increased the fine root length. Moreover, the carbon/nitrogen ratio of microbial biomass from control soil is a factor of 1.3 higher than that of the neighboring biochar compartment and roots in control soil can take up 1.2 times of the nitrogen compared to the biochar compartment. This demonstrates that microbes in untreated soil can mobilize more nutrients by a higher release of extracellular enzymes compared to in biochar-treated soil. In boxes without nitrogen fertilization, strong competition between plant roots and microorganisms for N became evident.

In conclusion, an enzyme-specific response with negative effects on hotspot area but positive effect on the timing of rhizosphere hotspots of β -glucosidase and acid phosphatase arose from the biochar treatment and supported plants' C investment fueling microbial activity and nutrient uptake.

P.44 (will also be presented as poster pitch)

ANALYSIS OF THE SOIL MICROBIOME IN RESPONSE TO THE APPLICATION OF RECYCLING PRODUCED FERTILISERS

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Phosphorus is an essential nutrient for all living organisms. In plants, phosphorus is required to support several biological processes, with deficiencies ultimately resulting in stunted growth. Hence, phosphorus is very important as an agricultural fertiliser. However, phosphorus is usually applied to soils in the form of inorganic fertilisers. With the world's finite phosphorus resources depleting and the population growing, innovation in terms of phosphorus recovery has become more important than ever in order to maintain global food supplies. ReNu2Farm is a North-Western European project funded by Interreg, which aims to efficiently recycle nutrients from varying sources (e.g. ash from sewage sludge), for use as fertiliser, and to encourage replacement of inorganic fertilisers by farmers. Part of this project is to assess the impact of these recycling-derived fertilisers on the soil microbiome, which provides key ecosystem services to our planet. Thus, in order to maintain these services, it is crucial that the recycling-derived fertilisers exhibit no adverse effects on soil microbial diversity or biomass.

Preliminary tests were conducted in greenhouse scale experiments through controlled pot trials using grassland soil planted with perennial ryegrass. The trial assessed 4 recycling-derived fertilisers (2 struvite and 2 ashes), as

well as a commercial inorganic fertiliser and a control (no phosphorus) for comparison. Sampling for microbiome analysis took place at five time points, once prior to P application, then 2, 4, 6 and 8 weeks after P application. DNA was extracted from the samples and sent to a sequencing company for analysis of bacterial 16S ribosomal DNA. The results will be used to inform a field trial beginning in late spring 2019.

P.45 (will also be presented as poster pitch)

FUNCTIONAL ANALYSIS OF PHOSPHATE TRANSPORTER *BnaPHT1;48* IN BRASSICA NAPUS

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Phosphorus (P) is one of the essential nutrients for plant growth and development. The total P content in arable land is high in China, but the bioavailability of phosphorus is extremely low. The PHT1 family of phosphate transporters is a high-affinity phosphate transporter family that play an important role in P uptake and allocation in plants. A phosphate transporter gene was cloned from *B. napus*, named *BnaPHT1;48*. The function of the gene in response to phosphorus stress was analyzed. The results showed that the open reading frame of *BnaPht1;48* was 1605 bp, encoding 535 amino acid. To determine the subcellular localization of this gene, we constructed *BnaPht1;48*:GFP fusion expression vector, which was transformed into tobacco leaves by Agrobacterium-mediated transformation. The protein was localized on the plasma membrane and nucleus. At the same time, in order to determine the expression pattern of the gene, we performed RT-PCR analysis of the different tissues of the gene at the seedling stage and the maturity stage. The results showed that the gene was induced by phosphorus deficiency in roots, basal ganglia, petioles and leaves, and the highest expression in roots. The gene was significantly up-regulated by phosphorus deficiency in both old and new leaves at maturity stage. In order to more clearly analyze the function of *BnaPht1;48*, we constructed *BnaPht1;48* overexpressing plants in *B. napus* and *Arabidopsis*, and analyzed the phenotype and found that: under the normal treatment, the phosphorus concentration in the shoots of the transgenic plants was significantly increased in *B. napus*. Under the low phosphorus condition, the phosphorus concentration in the roots was significantly higher than wild type. The concentration of Pi in *Arabidopsis* overexpressing plants was significantly higher than wild type. This study may provide a possible genetic approach for improving the P use efficiency of *B. napus*.

P.46

FACILITATED PHOSPHORUS ACQUISITION OF MAIZE IS DEPENDENT ON THE ROOT TRAITS OF ASSOCIATED FABA BEAN IN INTERCROPPING SYSTEMS

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Plants deal with phosphorus deficiency by changing the root morphology or strengthening the rhizosphere processes. How does these strategies affect adjacent plants in intercropping? Previous studies have proved that faba bean roots can release proton, acid phosphatase and organic acid to mobilize insoluble phosphorus in soils, however, there is still lack of study on how morphological parameters of faba bean roots affect the

phosphorus acquisition of maize. The objectives of the present study is to investigate the root morphological and rhizosphere differences among faba bean varieties and their effects on the growth and phosphorus uptake of associated maize, and to identify the mechanisms of efficient phosphorus acquisition in faba bean/maize intercropping.

A pot experiment was carried out to examine four faba bean cultivars, with difference in mobilizing soil sparingly soluble P and in root morphology, interacted with maize in three root interactive intensity (solid barrier, nylon mesh barrier and no barrier).

Results showed that acid phosphatase in rhizosphere soil of cultivar Lincan was 18.4% and 18.7% higher than that of cultivars Minqing and Zhangye. The citrate concentration in rhizosphere soil for cultivar Kangleniutabian was 2.1 times and 2.3 times as much as that of Minqin and Zhangye, and the malic acid concentration was 2.32 times and 2.31 times as much as those of Mingqin and Zhangye. The root length, root surface area and root volume of Lincan are all significantly higher than other cultivars. The rhizosphere processes of faba bean was positively correlated with not only P acquisition of faba bean itself, but also P acquisition of associated maize. The morphological parameters of faba bean roots was negatively correlated with P acquisition of associated maize.

The finding has shed a light on understanding the mechanism behind interspecific facilitation on P acquisition, but also have an importance in selecting a reasonable cultivar in intercropping.

P.47

THE EFFECT OF COPPER TOXICITY ON SYNERGISMS AND ANTAGONISMS BETWEEN NUTRIENTS IN GRAPEVINE PLANTS

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Copper (Cu) accumulation in soils can induce toxicity and nutrient imbalances in several plant species. The aim of this work was thus to evaluate the effect of Cu toxicity on two grapevine rootstocks, Fercal and 196.17, and to elucidate if intercropping with oat can alleviate grapevine Cu toxicity using hydroponic trials and rhizobox experiments.

The hydroponic trial revealed that Cu-induced root exudation was correlated with genes expression (*VvPEZ*-like); furthermore the ionome analysis revealed that both mono- and intercropped 196.17 rootstocks display a synergistic effect on Zn and Mn in the root tissues at high Cu concentrations. An increase of Zn and Mn in roots was also reported for the intercropped Fercal rootstock at high Cu concentrations while an antagonistic relation was observed for root Zn in the monocropped Fercal rootstock. The rhizobox experiments further confirmed these results showing a different nutrient concentration depending on the rootstock and on the soil characteristics. Indeed, Cu availability is shaped by rhizosphere processes, which depend on soil properties and/or the co-cultivation of different plant species. The soil-based experiments revealed that nutrient availability and dissolved organic carbon including root exudates differ depending on soil properties and the rootstock rather than on the cultivation system. In particular, we did not observe any apparent competition

between the two plant species in the alkaline soil; on the other hand, in the acid soil, the intercropping revealed a beneficial effect reducing the available Cu in the rhizosphere.

Our results revealed that Fercal rootstock is able to take advantage from oat, while 196.17 seems disadvantaged by the intercropping system. Yet, even though the intercropping system seems to be a valuable tool to counteract grapevine Cu toxicity, the application of this agricultural practice has shown to be species and mostly soil type dependent and should be evaluated for each rootstock.

P.48 (will also be presented as poster pitch)

CHARACTERIZATION OF DIFFERENTIALLY ABUNDANT BACTERIA IN RHIZOSPHERE OF FIELD GROWN BRASSICA NAPUS GENOTYPES-IMPLICATIONS FOR BREEDING

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Successful integration of an ideal rhizosphere microbiome through targeted plant breeding is key to harnessing existing and new positive plant-microbial interrelationships in cropping agro-ecosystems. Here we examine the composition of bacterial communities in the rhizosphere of diverse *Brassica napus* (canola) genotypes to identify bacterial taxa that preferentially associate with particular genotypes. Rhizosphere soils of sixteen diverse *B. napus* genotypes sampled weekly over a ten-week period in three replicates were analyzed using a 16S rRNA gene amplicon sequencing approach. The *B. napus* rhizosphere microbiome is characterized by diverse bacterial communities with thirty-two named bacterial phyla, almost one third of the currently ~92 named phyla. The most abundant phyla were Proteobacteria, Actinobacteria and Acidobacteria. Compared with a reference *B. napus* genotype a total of 167 bacterial genera were significantly more abundant in at least one different *B. napus* genotype and 114 were significantly less abundant out of the total 982 genera. We observed the highest number of total and genotype specific differentially more abundant genera in the most diverse *B. napus* genotype. Proteobacteria and Actinobacteria were the major contributors of the differentially abundant bacterial genera, accounting for 64 to 70% of the differentially abundant taxa in each *B. napus* genotype. Other main contributors of the differentially abundant genera included Bacteroidetes and Firmicutes (12 to 17% of genera). The differential abundance analysis highlights potential beneficial *B. napus* -bacterial interrelationships that could be beneficial and potential targets for direct or indirect selection within breeding programs. Here we have identified potential beneficial roles including bacteria associated with disease suppressive soil, anti-fungal properties and plant growth promotion. This approach of identifying genotype specific differentially abundant taxa and major contributing bacterial groups could be a starting point that leads towards integrating plant microbiome considerations in canola breeding programs.

P.49

EFFECTS OF BIOLOGICAL NITRIFICATION INHIBITOR 1,9-DECANEDIOL AND OTHER BNIS FROM RICE ROOT EXUDATES ON SOIL NITRIFICATION

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The application of biological nitrification inhibitors (BNIs) is considered an important new strategy to mitigate nitrogen losses from agricultural soils. 1,9-decanediol was recently identified as a new BNI in rice root exudates and was shown to inhibit nitrification in bioassays using *Nitrosomonas*. However, the effect of this compound on nitrification in soils remained unknown. In this study, three typical agriculture soils were collected to investigate the impact of 1,9-decanediol on nitrification and ammonia oxidizers in a 14-day microcosm incubation. High doses of 1,9-decanediol showed strong soil nitrification inhibition in all three agricultural soils. Moreover, the inhibition of 1,9-decanediol was superior to the widely used synthetic nitrification inhibitor, dicyandiamide (DCD) and two other BNIs, methyl 3-(4-hydroxyphenyl) propionate (MHPP) and α -linolenic acid (LN), in all three soils. The abundance of ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) was significantly inhibited by 1,9-decanediol addition across the three soils. 1,9-decanediol could also significantly reduce soil N₂O emissions, especially in acidic red soil. Moreover, synergistic effects of 1,9-decanediol and another BNIs SA from rice on soil nitrification inhibition were also observed. Our results provide evidence that 1,9-decanediol is capable of suppressing nitrification in agricultural soils through impeding both AOA and AOB. 1,9-decanediol holds promise as an effective biological nitrification inhibitor for soil ammonia-oxidizing bacteria and archaea.

P.50

PERFORMANCE OF TRANSPLANTED 'HONEYCRISP' APPLE TREES INCREASED BY BOTH IN VIVO- AND IN VITRO-PRODUCED ARBUSCULAR MYCORRHIZAL INOCULA (RHIZOPHAGUS INTRARADICES, FUNELIFORMIS MOSSEAE, CLAROIDEOGLOMUS ETUNICATUM, GLOMUS AGGREGATUM)

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Application of *in vivo*- and *in vitro*-produced mycorrhizal inocula in agricultural systems has become increasingly popular to improve crop performance. The goal of this study was to investigate the efficacy of *in vivo*- versus *in vitro*-produced MycoApply[®] inocula in container-grown apple trees. Commercially available MycoApply[®] mycorrhizal inocula containing four arbuscular mycorrhizal species (*Rhizophagus intraradices*, *Funeliformis mosseae*, *Claroideoglobus etunicatum*, *Glomus aggregatum*) and produced either *in vivo* (trade name MycoApply Endo) or *in vitro* (trade name MycoApply EndoMaxx) were applied to bare-root 'Honeycrisp' apple trees at a rate of 6,000 propagules per tree. Single trees were planted into 450 kg tote bags with calcined granular clay medium and grown under reduced nutrient and water supply. Each treatment contained 6

individual tree replicates. ANOVA analysis was used to determine statistical significance at a P level < 0.05. Both *in vivo*- and *in vitro*-produced inocula were effective in increasing mycorrhizal colonization, vegetative growth, relative chlorophyll content, photosynthesis, and chlorophyll fluorescence. Although there were no clear effects on root volume (measured by water displacement), noticeable differences in the nutrient content were observed between the leaves of inoculated trees and the untreated trees. Mineral nutrient content in the leaves increased (N, P, Mn) and decreased (Na, Al, Cu). This study confirmed equal efficacy of *in vitro*- and *in vivo*-produced mycorrhizal inocula to improve performance of apple tree as host plant.

P.51

SOIL PHOSPHORUS ACQUISITION BY COVER CROP SPECIES

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Cover crops or green manures may be included in crop rotations to enhance biological phosphorus cycling, and thereby improve phosphorus use efficiency. The objective of this study was to assess and quantify the relative ability of different cover crop plant species to acquire phosphorus from a soil that had received significant inputs of phosphorus fertilizer (Olsen P 21 mg P/kg). Eight plant species were investigated; including white lupin (*Lupinus albus*), blue lupin (*Lupinus angustifolium*), Russell lupin (*Lupinus polyphyllus*), pigeon pea (*Cajanus cajan*), chickpea (*Cicer Arietinum*), buckwheat (*Fagopyrum esculentum*), white clover (*Trifolium repens*) and *Tithonia speciosa* were grown in soil packed into rhizosphere study containers. These containers included a 20µm mesh to create a rhizoplane that separated bulk and rhizosphere soils. After 60 days, different forms of phosphorus in bulk and rhizosphere soils were determined using sequential extraction (fractionation), and organic anions exuded from roots at the rhizoplane were determined using capture by anion exchange membrane. Plant uptake decreased total phosphorus by an average of 8% compared with the original soil, and was greatest for white lupin (12%) and least for buckwheat (3%). Inorganic phosphorus depletion by plants mainly occurred from the moderately labile pool, while significant decreases were determined for moderately labile and stable forms of soil organic phosphorus. Total organic anion concentrations measured at the rhizoplane ranged from 1 to 13 µg/cm²/h. While citrate and malate were the predominant anions detected, depletion of soil inorganic and organic phosphorus fractions was most closely related to formate concentration in root exudates. The findings of this study revealed that short-term mobilization of soil phosphorus was greatest for white lupin and blue lupin, and these species will be subject to further mechanistic and practical investigation.

P.52

GRAMINEAE ROOTS AFFECT CH₄ SEQUESTRATION IN TROPICAL GRASSLAND SOILS

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It is well established that conversion of Amazon rainforest to cattle pastures turns the soils into emission sources of methane due to increased activity of methane producing over methane consuming microorganisms. Bad pasture management leads to 47% of Amazon pastures (80-100M ha) to exhibit some level of degradation, yet it remains unknown how pasture conditions impact methane fluxes. This research determined the effect of pasture management with liming (acidity correction) under plant coverage (rhizospheric effect) on soil methane fluxes. In a greenhouse study with soils sampled in southwestern Amazonia (S09° 54'58", W63° 02'27") from areas of primary rainforest, well-managed pasture, and degraded pasture. Soils were sieved and settled in pots, with four replicates each, for the treatments (1) control, (2) acidity corrected, (3) plant added, and (4) plant added and acidity corrected. To mimic field conditions the plant was *Brachiaria brizantha* cv. *Marandú*, the dominant pasture grass in Brazil, and acidity was corrected by calcium carbonate addition. The methane fluxes were measured regularly for 115 days using static chambers and an infrared gas analyzer (UGGA, Los Gatos Research). After that, rhizosphere soil samples were collected for DNA extraction, and prokaryotic diversity was determined by amplicon sequencing of the 16S rRNA gene (515F / 806R) on the Illumina Miseq v3 platform. Soil acidity correction by liming reduced methane sequestration in bulk soils regardless of land use. The presence of the plant increased methane sequestration by $27.4 \pm 7\%$. These changes were associated with a decrease in the relative abundance of the methanogens *Methanocella* sp. (-75%), *Methanobacterium* sp. (-66%), and the potentially methanogenic group Bathyarchaeota (-63%) in the rhizosphere soil from well-managed pasture. Our results indicate that changes in pasture management, such as acidity correction and maintenance of good grass coverage, may increase the methane sink capacity in tropical grassland soils

P.53

ENHANCEMENT OF RHIZOSPHERE CITRIC ACID AND DECREASE OF NO₃⁻/NH₄⁺ RATIO BY ROOT INTERACTIONS FACILITATE N FIXATION AND TRANSFER

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Aims Cereal can stimulate legume N fixation through competition for soil mineral N. In addition, organic acids in the rhizosphere can mobilize external nutrients, which may help nodulation. However, how much NO₃⁻ and NH₄⁺ competition and organic acids in the rhizosphere of intercropped crops contribute to N fixation remains unclear and requires study.

Methods Field and greenhouse experiments were conducted to quantify effects of N competition and organic acids on N fixation in a maize/alfalfa intercropping system.

Results Intercropping increased nitrogen derived from the atmosphere (%Ndfa), nodules number, and leghemoglobin content by 43.66%, 85.53%, 141.05%, respectively, leading to significantly improved total N uptake and yield, compared to monoculture. The improved %Ndfa was not only due to the depletion of NO_3^- , but also significantly correlated with the decrease of $\text{NO}_3^-/\text{NH}_4^+$ ratio and the increase of citric acid in rhizosphere of alfalfa, which could mobilize soluble resources for N fixation under no N addition. Overall, 15.4%-21.5% of N fixed by alfalfa was transferred to associated maize, and the improved N fixation enhanced N transfer.

Conclusions Our findings provide a mechanism for how root interactions facilitate N fixation, highlighting the importance of $\text{NO}_3^-/\text{NH}_4^+$ ratio and citric acid in nutrient mobilization for N fixation.

P.54 (will also be presented as poster pitch)

STRUCTURE OF NITROGEN-TRANSFORMING RHIZOBACTERIAL COMMUNITIES UNDER DIFFERENT LAND USES

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Microorganisms, in the course of their growth and metabolism, interacts successively in re-cycling of nutrients such as Nitrogen (N). This element takes different reduced and oxidized states in soils. Rhizobacterial communities may play a crucial role in these transformations. However, our knowledge of how physical and chemical properties of Ñadi soil (Aquands) modulates bacterial communities in pastures and forests is still poor. The Ñadi soils (Aquands) are located in southern Chile (38°30'–43°00' S) and dominated by grasslands that support the major livestock production and, consequently, one of the most relevant grassland agro-ecosystems. Moreover, these soils present extreme and contrasting water content conditions depending on the water table height. Here, we investigated the influence of physical and chemical properties of Ñadi soil on Nitrogen-transforming bacterial communities' structure under different land uses. Sizes and diversities of bacterial communities were studied using Illumina MiSeq platform to sequence 16S rRNA gene and denaturing gradient gel electrophoresis (DGGE) for analysis *amoA* and *nirK* genes as bacterial molecular markers of ammonia oxidation and nitrite reduction, respectively. DNA extractions were taken from soil samples (Naturalised Pasture-PN, Evergreen Native Forest – *Nothofagus dombeyi*– BN1 and Conifer Native Forest – *Fitzroya cupressoides*- BN2) during winter and summer. Our preliminary results showed that pH, organic matter and gravimetric water content have a significant influence on bacterial communities. Moreover, DGGE showed a higher diversity of bacterial communities harboring *nirK* and *amoA* genes in the forest samples (BN1 and BN2) during the winter season than pasture samples (PN). This preliminary study contributes to understanding the N-transforming bacterial communities, which are also relevant considering the potential consequences of climate change in southern Chile, increase in soil temperature and reduction in rainfalls on water and temperature dynamics as well as microbiological activity on these soils.

P.55

ORGANIC ANION EXUDATION OF FIELD GROWN CROPS MORE RESPONSIVE TO NITROGEN AVAILABILITY THAN PHOSPHORUS DEFICIENCY

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The experimental aim was to measure organic anion exudation from the rhizosphere of field grown crops ranging in acid soil sensitivity. Root exudation of some organic anions has been shown to increase in plants suffering phosphorus deficiency and aluminium toxicity. However, exudation has also been shown to be responsive to nitrogen availability. Cereals (*Triticum aestivum*, *Secale cereale*) and forage legumes (*Medicago sativa*, *Trifolium subterraneum*, *Ornithopus sativus* and *O. compressus*) were grown in 40m² plots (three replicates) with or without lime. Liming increased topsoil pH (0 - 10 cm) from 4.6 to 6.5 (CaCl₂), increased nitrogen and phosphorus availability three fold and significantly reduced aluminium availability.

Exudation of organic anions was consistently greater in the rhizosphere of plants grown in limed plots, particularly for forage legumes. Significant quantities of citrate (11 – 84 µmol g⁻¹ root dry mass), malate (11 – 76 µmol g⁻¹ root dry mass) and malonate (21 – 95 µmol g⁻¹ root dry mass) were detected in the rhizosphere of *Ornithopus* cultivars and were two to four times greater in limed plots. The amount collected from *T. subterraneum* and *M. sativa* roots were significantly less than for *Ornithopus* species, however exudation in limed plots was three to five times greater for *T. subterraneum* and ten times greater for *M. sativa* than in control plots. For cereals, malate was the main organic anion detected (≤ 12 µmol g⁻¹ root dry mass) and was generally only found in plants from limed plots.

Exudation of organic anions was shown to be greatest where liming had raised pH and increased nutrient availability. Nitrate has been positively correlated with increases in synthesis of organic anions and seems to be a greater driver for exudation than either phosphorus deficiency or aluminium toxicity, even in species reportedly sensitive to these environmental stresses.

P.56

BACILLUS ARYABHATTAI SP1016-20: A POTENTIAL BIOCONTROL AGENT FOR SALINE AGRICULTURE

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Saline agriculture and crop cultivation of halophytes represent alternatives for the reclamation of salinized soils and for the management of irrigation water. However, halophytes are also exposed to phytopathogens that negatively affect productivity, organoleptic characteristics of edible parts and market value. Halotolerant plant growth promoting rhizobacteria (PGPR) with biocontrol effect can be used for the dual purpose of preventing fungal disease and attenuating saline stress effects. The objective of this work was to isolate autochthonous rhizobacteria with biocontrol features with plant-growth promoting potential for the crop cultivation of *Salicornia ramosissima*.

Bacteria from the rhizosphere of wild *S. ramosissima* specimens collected at *Ria de Aveiro*, a tidal estuarine system on the NW coast of Portugal, were isolated and characterized in terms of Gram, motility, salt tolerance

and biocontrol traits. One Gram-positive motile isolate that tested positive for all biocontrol traits was identified as *Bacillus aryabhatai* by 16S rRNA gene sequencing. The inoculation of *S. ramosissima* seeds with *B. aryabhatai* SP1016-20 attenuated the negative effect of salinity on the germination efficiency. At the highest tested salinity (30 PSU) the final germination efficiency of inoculated seeds doubled in relation to non-inoculated controls.

Although the defined mechanisms involved in the stress attenuation and in the biocontrol effect could not be discriminated, the results highlight the potential of *Bacillus aryabhatai* SP1016-20 as a plant-growth promoting agent for the crop cultivation of *S. ramosissima* and contribute to the scientific basis of PGPR-assisted crop cultivation of halophytes in saline soils and estuarine sediments.

P.57 (will also be presented as poster pitch)

ROOT RESPONSES TO SODIC-ALKALINE CONDITIONS AND THEIR INFLUENCE ON PLANT GROWTH

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Much of the world's arable land is classified as sodic, meaning that crops cultivated in these regions are exposed to high levels of carbonates and alkalinity. High pH results in altered bioavailability of nutrients and even renders some ions toxic. Current interventions require significant costs and labour input which are not sustainable solutions, particularly in the face increasing need for greater food security. An attractive alternative would be to enhance the crop's own battery of tolerance mechanisms deployed under these harsh conditions such that they can overcome the detrimental effects of hostile soils. These mechanisms may include innate root responses such as altered root morphology or exudation of various compounds to condition the rhizosphere for optimal growth.

Several bread wheat lines were selected for diversity in alkaline tolerance. These were characterised by variation in root length between lines grown in neutral and high pH conditions. Other phenotypic responses will be compared to genetic data available for these lines to help define regions of the genome that provide better resistance to sodic soils. The mechanisms of tolerance in these lines is not known and we hypothesise this may be due to the ability of the roots to acidify the rhizosphere, enhancing the bioavailability of vital nutrients. Novel pH indicator techniques using bromocresol purple have been developed to correlate rhizospheric acidification with alkalinity tolerance. As well as acidification capacity, alterations in root morphology have been examined. An apparent increase in lateral root production and an unusual root kinking phenotype have been observed. This kinking phenotype is being characterised to determine whether there's correlation to the degree of tolerance. This study aims to expand our fundamental knowledge of stress responses, which will inform breeders of valuable traits to provide superior lines capable of utilising their own rhizospheric processes to overcome nutrient deficiencies.

P.58

SOIL ENZYMATIC NITROGEN TRANSFORMATION IN RESPONSE TO VARYING N TREATMENTS ACROSS FOUR DIVERSE BRASSICA NAPUS (CANOLA) LINES.

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Nitrogen (N) is an important plant nutrient, but it can be limiting in the soil. Improving N use efficiency (NUE) in crops is an important goal in sustainable agriculture. The study objective was to determine rhizosphere N transformation rates between diverse canola (*Brassica napus* L.) varieties grown under varying urea fertilizer application rates. Two diverse canola parent varieties and two hybrid varieties were grown on a Dark Brown Chernozem in Saskatchewan, Canada. Root-associated soils from the 5-6 leaf stage and flowering, were analyzed for urease, ammonium oxidase, nitrate-N ($\text{NO}_3\text{-N}$), ammonium-N ($\text{NH}_4\text{-N}$); aboveground plant and seed N was determined. Urease activity and $\text{NH}_4\text{-N}$ were higher at flowering versus the 5-6 leaf stage, while canola growth stage had an opposite effect on ammonium oxidase activity and $\text{NO}_3\text{-N}$. Ammonium oxidase and urease activities decreased with increasing urea rates; while $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ increased with urea rates. At flowering, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ was lowest under the hybrid H151857, and highest under the parent variety NAM-17, compared to other genotypes. Aboveground percent N was higher at the 5-6 leaf stage, and plant and seed N increased with urea rates. By characterizing soil N transformations, this research will advance our knowledge in improving canola NUE.

P.59

SECURING ECOSYSTEM SERVICES WITH ABOVE AND BELOWGROUND BIODIVERSITY

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With the continued and intensifying impact of anthropogenic activities on ecosystem processes, there is growing concern as to how our ecosystems will function to meet society's future needs. Addressing the concerns as to how we might manage ecosystems for securing ecosystem services into the future requires determining how ecosystems may respond under global change scenarios and the underlying dynamic interactions within ecological communities. Here I present evidence that crop diversification can enhance soil biodiversity that can be instrumental for promoting multiple soil ecosystem services. Identifying the links between crop diversification and soil biodiversity for promoting ecosystem productivity provides promising insights as to how crop diversification may be used to engineer healthy belowground soil biomes that are needed to secure the ecosystem services our society depends upon.

P.60

LABILE SOIL ORGANIC MATTER POOLS WITHIN RHIZOSPHERES OF GRASS SPECIES USED TO AMELIORATE DEGRADED SOILS UNDER A SOUTH AFRICAN SEMI-ARID ENVIRONMENT

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Uncontrolled stocking in communal livestock grazing areas of the semi-arid North-West province in South Africa leads to breakdown of soil structure and erosion. Planting of grass has widely been used as an effective soil erosion control technology that also ameliorates soil structure. This study evaluated the labile pools of soil organic matter (light fraction and microbial biomass) in the rhizospheres of three grass species *viz* *Vetiveria zizanioides* (vetiver grass, VG), *Cynodon dactylon* (kikuyu grass, KG) and *Themeda triandra* (a natural fallow grass, FG) that had been grown in adjacent plots for over 21 years. The above ground biomass of the grasses at the time of study was estimated to be 5241, 2786 and 2017 kg ha⁻¹ in plots with VG, KG and FG respectively. Both root mass density and root length density in the top 40 cm depth were in the order VG>KG>FG. The microbial biomass C and N measured using the fumigation extraction method was consistently higher in plots under KG. The ANOVA showed significant differences ($p<0.05$) among grass species in both the light fraction and microbial biomass within the profiles of the plots. Even though plots with VG had a superior above ground biomass, root mass and length density than KG and NG, the labile pools of organic matter were in the order KG>VG>NG. Since the turnover of microbial biomass and the colloidal and soluble organic compounds from root exudates are the principal inputs of the labile organic matter fraction, the result could suggest that the quantity and quality of this fraction were higher under KG than VG and NG. The significant positive correlations between particulate organic matter, microbial biomass and soil structural properties assessed in the study underscore the importance of the labile soil organic matter fraction in enhancing of soil quality in low active clay soils.

P.61

ALUMINUM TOXICITY COULD BE MITIGATED WITH BORON BY ALTERING THE METABOLIC PATTERNS OF AMINO ACIDS, CARBOHYDRATES, AND ASCORBATE SYNTHESIS IN TRIFOLIATE ORANGE

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Aluminum toxicity is a major limiting factor for plant productivity. Boron could mitigate aluminum toxicity in many plant species. However, the exact mechanisms underlying boron on aluminum-induced alteration on root metabolites are poorly understood especially in the trifoliolate orange which is important rootstock in China. Therefore, hydroponics experiment was conducted to explore the mechanisms how boron mitigates aluminum toxicity in roots of citrus by metabolomics. Results showed that the 17 amino acids and 8 sugars were up-regulated in aluminum-treated roots, mainly histidine, cycloleucine, asparagine, citrulline, raffinose, and trehalose. While significant down-regulation of aspartic acid, isoleucine, glutamic acid and 6 sugars were indicated under aluminum stress. Aluminum-induced a decrease of 9 organic acids, especially L-malic acid, citric acid, and threonic acid. Interesting, in the presence of aluminum, boron application decreased the contents of

asparagine, cycloleucine, citrulline, and histidine as well as *myo*-inositol, raffinose, galactinol, and 3, 6-anhydro-d-galactose. However, there was no obvious difference in organic acids contents in aluminum-stressed roots treated with boron. More importantly, boron decreased the aluminum-stimulated ascorbate synthesis by down-regulated ascorbate synthesis-related metabolites in the L-galactose pathway such as D-mannose-1-P, L-galactose-1-P, L-galactose, and L-galactono-1, 4-lactose. Meanwhile, boron alleviated some of the toxic effects of aluminum by decreasing redox states of ascorbate and enzyme activities involved in ascorbate-glutathione cycle. Conclusively, our results show that boron regulates the metabolic patterns of amino acids and carbohydrates, and reduces aluminum toxicity. Nevertheless, boron addition didn't affect the aluminum-induced changes in the metabolic modes of organic acids. Moreover, boron protects roots against aluminum-induced oxidative stress possibly by reducing metabolites accumulation in the L-galactose pathway of ascorbate synthesis and regulating ascorbate-glutathione cycle. This study may provide physiological insights into comprehending the alleviate mechanisms of aluminum toxicity in plants treated with boron.

P.62

ALLOCATION OF PHOTOSYNTHESIZED CARBON FROM RICE INTO RHIZOSPHERE SOIL AGGREGATES DEPENDS ON LONG-TERM FERTILIZATION REGIMES

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Rice transfers carbon (C) to soil throughout the growing season by the process of rhizodeposition. The proportion of C in root exudates that remains in soil should be greater in organically fertilized soils, which support larger microbial biomass, than in soils receiving no fertilizer or inorganic fertilizer. This study examined the allocation of rhizodeposited C in rice-soil systems under three fertilizer regimes. Rice was grown in soils with an eight year history of no fertilizer, NPK fertilizer or NPK fertilizer plus cattle manure, and was pulse-labeled with ¹³CO₂ at the tillering stage. In the organic C pool of rhizosphere soil, there was 2.9 times less ¹³C in the unfertilized treatment, and 1.5-fold lower ¹³C in the NPK fertilizer treatment than in the NPK fertilizer plus cattle manure treatment. Rhizosphere soil was sieved to collect aggregates (<0.25 mm to >2 mm). In microaggregates (<0.25 mm), there was 87% greater ¹³C concentration in the NPK fertilizer treatment and 135% more ¹³C in the NPK fertilizer plus cattle manure treatment than in the unfertilized control. There was more ¹³C enrichment in the >2 mm aggregate fraction than in microaggregates (<0.25 mm) of the NPK fertilizer plus cattle manure treatment. It appears that the NPK fertilizer plus cattle manure treatment was most conducive to retain C from root exudates in the rice-soil system.

P.63

FIELD TRANSCRIPTOMICS REVEALS A GENE-COEXPRESSION MODULE THAT REGULATES MYCORRHIZAL FORMATION AND FUNCTIONING

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Arbuscular mycorrhizal (AM) fungi form symbiotic associations with 75% of land plants. The fungi deliver mineral nutrients, especially phosphate, to the host plant, and in exchange, the host supplies organic carbon. Various genes responsible for mycorrhizal formation and nutrient exchange have been identified in the laboratory model systems, but how the plant and fungal symbionts optimize the association in the field is largely unknown. Maize roots were collected across five states in the US (66 plants) and across seven prefectures in Japan (194 plants). mRNA was purified from the roots, sequenced on the illumina platform, and mapped to the maize genome, and gene-coexpression network analysis was performed. Unmapped reads were assembled *de novo* and mapped to AM fungal sequences that were identified from the contigs with blastx searches against the AM fungal genome sequences available in the database. The network analysis revealed that the majority of the genes involved in mycorrhizal formation and functioning were enriched in a single co-expression module, that is, a 'mycorrhizal module'. Eigengene values (principle component 1 scores) of the module were correlated not only with transcript abundance of the plant genes encoding mycorrhiza-specific transporters and enzymes for nutrient exchange but also with those of the fungal genes encoding ribosomal RNA (biomass marker), a phosphate exporter, and a vacuolar phosphate exporter that are responsible for fungal phosphate delivery. These results suggest that relative expression levels of the genes in the mycorrhizal module are tightly regulated across environments, but their absolute expression levels are flexibly modulated in response to fungal abundance and functioning levels of the fungi.

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MULTIFACTORIAL INFLUENCE OF LONG-TERM AGRICULTURAL MANAGEMENT PRACTICES ON RHIZOSPHERE MICROBIOTA ASSEMBLAGE AND PERFORMANCE OF LETTUCE.

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Agricultural management practices play a significant role in the alteration of soil biological properties. We conducted an exploratory research using a multidisciplinary approach aimed to investigate the effects of long-term agricultural management practices on the plant performance and rhizosphere prokaryotic (*Bacteria* and *Archaea*) community composition. We conducted growth chamber experiments with lettuce (*Lactuca sativa* L.) cultivated in soils from a long-term field trial established in 1992 (Bernburg, Germany). Soils were collected

from fields after harvest of two different crops, namely, wheat and rapeseed grown under various tillage practices (mouldboard plough vs. Cultivator) and N-fertilization strategies (Standard intensive vs. 50% reduced extensive). As obtained by RT-qPCR analysis, N-fertilization strategy showed a significant effect on the expression intensity of selected genes of lettuce cultivated in the different soils. This was observed in both plough and cultivator soils and was independent of the last crop in the field (wheat or rapeseed). Our results showed higher shoot biomass in plants grown in soils under reduced N-fertilization intensity (Ext) compared to standard N-fertilization intensity (Int). High-throughput sequencing of bacterial 16S rRNA genes and fungal internal transcribed spacer fragments amplified from total community DNA showed effects of the tillage practice, the last crop in the field and N-fertilization strategy on the soil and rhizosphere microbial community compositions which resulted in a number of differentially abundant taxa. For instance, a higher relative abundance of several potential plant growth promoting genera like *Pseudomonas* and *Flavobacterium* was observed in the rhizosphere of lettuce grown in soil under cultivator tillage practice compared to plough tillage. Our results revealed that soil legacies induced by multiple factors in long-term agricultural management practices shaped the soil microbial communities and influenced the rhizosphere microbiota assemblage as well as lettuce plant performance.

P.65

IS BARLEY RESISTANCE TOWARDS *BLUMERIA GRAMINIS* INFLUENCED BY ITS RHIZOSPHERE MICROBIOTA?

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Agricultural management is assumed to be an important factor shaping soil microbial communities. We hypothesize that the rhizomicrobiota of barley grown in soils under different long-term management or fertilization will harbor different microbial communities. Therefore, barley rhizosphere (BBCH55) and bulk soil sampled from a long term field experiment (LTE) with four different agricultural managements (mouldboard plough (MP) and conservation cultivator tillage (CT) with standard N-fertilization and pesticide application (I) or reduced N-fertilization without pesticides (E)) was investigated by amplicon sequencing of 16S rRNA gene fragments amplified from total community (TC)-DNA. Significant differences in the prokaryotic community composition were revealed between the soil managements MP and CT, but not between the fertilization treatments. In order to test our hypothesis, a greenhouse experiment was conducted with the barley cultivar Golden Promise grown in these four differently managed soils and in a standard substrate as control until BBCH13 and subsequently infected with model pathogen *Blumeria graminis* f. sp. *hordei*. Relative gene expression profiles of the defense-related genes *PR1b* and *PR17b* were determined before and 24 h after infection and expression tended to be higher in all infected samples compared to the uninfected samples, with a significant difference for plants grown in MP-I soil. The amount of fungal hyphae was determined in a detached leaf assay and no differences were revealed for plants grown in field soil which all had significantly lower infection rates compared to the plants grown in standard substrate. Our results indicate that the rhizomicrobiota of barley grown under field conditions was shaped by the agricultural soil management. Whether management dependent differences under greenhouse conditions were less pronounced is presently

investigated by amplicon sequencing of 16S rRNA gene fragments obtained from TC-DNA of rhizosphere and bulk soil. Managing the soil microbiome could be a new approach to enhance crop resistance.

P.66

IMPACTS OF MOISTURE AND UREA ON N₂O EMISSIONS AND MICROBIAL ABUNDANCES IN ORCHARD SOIL MICROCOSMS

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Nitrous oxide is a major greenhouse gas that contributes to climate change and stratospheric ozone depletion. Agricultural soils are a major source of anthropogenic N₂O emissions. The two main N₂O generating processes from soil are microbial nitrification and denitrification. The goal of this study was to evaluate the effect of varying soil moisture and urea fertilization rates on N₂O emissions and microbial nitrifying and denitrifying population abundances from a perennial orchard soil sampled at two soil depths (5-15 and 15-30 cm). Soil was collected from a drip-irrigated apple orchard in the semi-arid Okanagan Valley, British Columbia, Canada and dried. The soil is classified as a sandy loam with 59.7 % sand, 29.7 % silt, and 10.6 % clay. Microcosms containing 50 grams of dried soil from each depth were subjected to varying moisture content (0, 30, 50 and 80 %) or nitrogen content (0, 25, 50 and 75 mg of urea/ 50 g dry soil) with four replicates of each treatment. N₂O emissions were measured on days 0, 1, 3, 5, 7 and 9 and DNA was extracted on days 0 and 9. Bacterial and archaeal *amoA* genes coding for ammonia monooxygenase, the first enzyme in the nitrification pathway, and bacterial *nirS* and *nosZI* genes coding for nitrite reductase and nitrous oxide reductase, respectively, were quantified by droplet digital PCR and correlated to nitrous oxide emissions and soil properties to understand the role of microbial processes in N₂O emission. The long-term aim of this research is to develop best management practices for orchardists to minimize N₂O emissions.

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SOLUBILIZATION MECHANISM OF NON-AVAILABLE POTASSIUM AND CESIUM OF WHITE LUPIN UNDER POTASSIUM-DEFICIENT SOIL

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It has been well known that white lupin adapts to phosphorus-deficient condition by modifying their root architecture and secreting metabolites such as organic acids to solubilize insoluble phosphate compounds. In our previous study, we found that white lupin accumulated high concentration of cesium in its shoot under potassium-deficient conditions. Moreover, cesium and potassium concentrations in leaves of soybean increased by mixed cropping with white lupin under potassium-deficient condition. These results suggest that in the potassium-deficient soil, white lupin can solubilize non-available potassium and cesium in rhizosphere soil. The objective of this study is to elucidate the mechanisms of the potassium-deficiency-induced solubilization of potassium and cesium in rhizosphere soils of white lupin.

White lupin (*Lupinus albus* L. cv. Kievskij) were grown in pots filled with three treatment soils (control, phosphorous-deficient, and potassium-deficient). pH of each soil was adjusted to 6.0 by using calcium carbonate. After cultivation of plants in growth chamber (22°C) for 44 days, inorganic element concentration in plants (HNO₃/H₂O₂ digestion) and soils (1M ammonium acetate extraction) was measured by Inductively Coupled Plasma-Mass Spectrometry. Net increase of available potassium in pots was calculated.

Cesium concentration in plants increased when growing in potassium-deficient treatment. In addition, the net increase of available potassium in soils also increased in both potassium and phosphorous deficient treatments. These results suggest that in soils in these treatments, white lupin can dissolve non-available potassium. We will analyze the dynamics of cesium in rhizosphere soil and metabolites in root-exudate.

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LEAF SENESCENCE, SHOOT AND ROOT GROWTH OF BARLEY AFFECTED BY SMALL MOLECULES UNDER NITROGEN DEFICIENCY

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Leaf senescence is a highly physiological controlled process, in which nitrogen (N) resources were translocated from older to younger leaves. Stress conditions, like N deficiency or dryness, induce earlier senescence with inefficient recycling processes and considerable yield losses. The use of Small Molecules (SM) as active substances offers an innovative approach to enhance the N use efficiency by targeting N uptake and transposition processes within the plant. Therefore, the aim of this study was to identify substances with positive effects on rooting, shoot growth and leaf senescence under N deficiency.

For this, spring barley plants were grown hydroponically in Hoagland solution for 32 days under high N (HN) and low N (LN) conditions, supplied with 7 and 0.7 mM, respectively. The tested SM Isonicotinamide (INAD) and Monoethanolamine (MEA) were applied to LN conditions in two concentrations of 0.1 and 10 µM. Senescence of the primary leaves was regularly evaluated using the photosynthesis-parameter photosystem II efficiency. After 32 days shoot and root parameters were measured.

The application of INAD and MEA under N deficiency delayed the primary leaf senescence by 4 % at 0.1 µM in each case as well as 20 and 4 % at 10 µM, compared to LN. Shoot dry mass was improved by 2 – 8 %, while the effect on total root length and root dry mass was even higher (up to 13 %). In both, INAD and MEA treatment the root to shoot ratio tended to increase. However, delayed leaf senescence did not affect the mean root diameter and specific root length.

In our attempt, the addition of SM under LN conditions extended not only the “stay green phase”, but also enhanced the shoot and root growth in trend. Furthermore, higher root to shoot ratios indicate an intensified focus on rooting in these treatments.

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ARYLSULFATASE ACTIVITY IN PLANT RHIZOSPHERE

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Sulfur deficiency is an actual problem in European region, mainly because of lower sulfur inputs in soil due to the atmospheric depositions. However, plants have different mechanisms to solve this problem. One of these mechanisms is increase of the arylsulfatase activity, which helps to the minealization of organic sulfur compounds in soil. Three stated hypothesis are following: i) sulfur deficiency increase the arylsulfatase activity, ii) with different plant species appears different arylsulfatase activity in the rhizosphere, iii) different fertilizing systems will have influence on arylsulfatase activity.

Experiment was established with three different plants: Spring rape (*Brassica napus*), Spring wheat (*Triticum aestivum*) and white lupine (*Lupinus albus*). These crops were grown in novel rhizoboxes, which allows to investigate the soil in different distances from the root surface. As the growing substrate, cambisol and chernozem from 2 long term field experiments was used. Furthermore, soils from three fertilizing treatments of mentioned experiment were evaluated: i) not fertilized control, ii) sewage sludge, iii) farmyard manure. The amount of 330 kg N/ha per 3 year crop rotation was applied in the form of sewage sludge or farmyard manure, respectively. Arylsulfatase activity was measured in the distance 1, 4 and >6 mm from the root surface, using the method based on the releasing of p-nitrophenol after incubation.

Similar tendencies were observed at both studied soils. Organic fertilizing increased the arylsulfatase activity at all three crops, where the sewage sludge treatment resulted in the highest arylsulfatase activity at wheat and farmyard manure resulted in highest arylsulfatase activity at rape and lupine, respectively. Arylsulfatase activity usually decreased with increasing distance from the roots, but the results were not statistically significant.

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THE EFFECT OF COPPER TOXICITY ON THE UPTAKE OF PHOSPHORUS IN CUCUMBER PLANTS

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Due to the arbitrary use of cupric fungicides in the last century, copper has accumulated in the soil. The concentrations exceed the European safety limits of risk assessment for copper in soil and this may cause toxicity in plants. Scientific knowledge about the interaction between copper and other nutrients is still scarce, also the impact of copper on the mechanisms underlying mineral nutrients uptake at root level. These aspects might be of paramount importance in the case of phosphorus, which is an essential macronutrient for all plants species. In this work hydroponically grown cucumber plants were used to investigate the influence of different copper concentrations (*i.e.* 0.2, 5, 25 and 50 μ M) on i) the phenotypic traits of plants, particularly at root level, ii) the nutrient content in both root and shoot, iii) the phosphorus uptake activity at both biochemical and molecular levels. The data display significances under elevated copper concentrations: the growth of shoot and root resulted stunted, and the net phosphorus uptake diminished. Furthermore, two phosphorus transporter

genes were up-regulated at the highest concentration: *CsPT1.4* showed a quick response, whereas *CsPT1.9* might be involved in a long-term response to the toxicity. Overall, these results strengthen our hypothesis that high copper concentrations in the growth medium can limit the uptake of phosphorus, most likely *via* a direct action on the uptake mechanisms (e.g. transporters) and/or by compromising the integrity of the root plasma membrane.

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UNDERSTANDING ROOT WATER UPTAKE AND SOURCE PARTITIONING: HIGH-RESOLUTION STABLE ISOTOPES MEASUREMENTS AND PLANT HYDRODYNAMICS

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Plant water use investigations using stable isotopes of hydrogen and oxygen ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) as a tracer have shed new light on ecohydrological processes in the rhizosphere. Such work has shown that plants rarely make use of precipitation and often use resident water bounded to the soil matrix. These “ecohydrological separation” observations to date have been done in open field conditions at low spatial-temporal resolution. Most importantly, these investigations have not yet considered plant water status. Here, we present a new controlled experiment using a 2 m³ lysimeter planted with a small willow tree (*Salix viriminalis*) to explore how plant water status affects water source partitioning and uptake. We combine high resolution sampling of tree and soil water isotopic composition with measurements of tree hydrodynamics (sap flow rate and stem radius change, using sap flow sensors and diameter dendrometers respectively), soil matric potential and climate data. Our results show a strong relationship between tree water deficit and soil matric potential and corresponding source water uptake. This coupling results in a dynamic root water uptake with changes in tree water source partitioning during the seven-week experiment. During a period where plant water status was not in deficit, xylem isotopic composition had equal contributions from soil water present throughout the 2 m soil profile. During a period of intermediate tree water deficit, this changed to shallow soil water dominance. During a period of strong tree water deficit, deep soil water sources dominated root water uptake. These data show that tree water status is important for understanding how plants explore available water resources in the subsurface. By adopting more robust measurements of plant hydrodynamics, we believe that significant advances can be made in understanding root water uptake and source water partitioning, and disentangle complex tree water interactions occurring in the rhizosphere.

P.72

MICROBES OF THE COFFEE RHIZOSPHERE - SITE AND MANAGEMENT IMPACTS

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The root microbiome of Central American coffee trees was studied from four different sites experiencing different annual temperatures and precipitation level, sampling from plot grown conventionally (under full sun) and under agroforestry management (with shade trees). Total community DNA was separately extracted from the rhizosphere roots from four trees sampled from each site/management pair and terminal restriction fragment length polymorphism analyses, and pooled for next generation sequencing (Illumina) of fungal and bacterial ribosomal RNA amplicons. Community profiles were analysed for site and management effects and were also correlated to environmental parameters. Major taxa of both bacteria and fungi varied with site locations, but were not impacted by management system type, although microbial diversities were. Core bacterial genera included *Dokdonella*, *Acidobacterium*, *Nitrosovibrio*, *Rhodoplanes*, *Edaphobacter*, *Burkholderia* and *Koribacter*. Fungal communities were much more variable between sites than were bacterial communities. The only two fungal genera that were abundant in all sites were *Xylomyces* and *Knufia*. Network analyses were used to explore the interrelationships between bacterial and fungal taxa in these rhizosphere communities.

P.73

CHANGES OF SOIL MICROBIAL AND PHYSICOCHEMICAL ECOLOGY RESULTED FROM LONG TERM APPLICATION OF ORGANIC FERTILIZER IN CUCUMBER MONOCULTURE SOIL IN NORTH CHINA

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Monoculture of cucumber without crop rotation in greenhouse is very common in North China, and leads to serious growth obstacle and yield loss. Application of manure and compost is one of the major practices that farmers use to reduce the loss. In this work we tried to investigate how this practice changed soil microbial and physicochemical ecology. Total of 18 samples were collected from cucumber rhizosphere and bulk soil in commercial greenhouses with 1, 3, 7, 15 and 25 cropping years. The soil physicochemical analyses demonstrated that all tested characters significantly increased after long term monoculture, referred by the data of soil organic matter (SOC) (from 7.84 to 67.17 g·kg⁻¹), total nitrogen (TN) (from 1.35 to 8.54 g·kg⁻¹), total phosphorus (TP) (from 0.014 to 0.243%), soluble nitrogen (SN) (from 187.21 to 1295.26 mg·kg⁻¹) and electrical conductivity (EC) (from 42.5 to 1632 μs·cm⁻¹). Soil fungal and bacterial community was analyzed by qPCR and high-throughput sequencing. The microbial amount increased with the cropping years, however, microbial diversity decreased. Further correlation analysis showed that the number of OTUs was negatively correlated with EC value, suggesting that the diversity reduction might result from the soil salinity. Besides, the abundance of potential pathogenic fungi *Fusarium* in Y25 samples was lower than that in Y3 samples, while beneficial bacteria *Bacillus*, *Pseudomonas* and *Streptomyces* had high abundance, and these bacteria positively correlated to nutrient accumulation in the soil, like TN and SOC. In a conclusion, continuous application of manure and compost in 25 years monoculture soil increased the soil nutrient and salinity, thus resulted in the reduction of

soil microbial diversity, but increased some beneficial bacteria and suppressed some pathogenic fungi. These results gave us some clues on the manipulation of soil ecology to keep soil quality.

P.74

SOIL MANAGEMENT AND MICROBIAL DIVERSITY IN DUTCH AGRICULTURAL SOILS

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Several case studies were developed to investigate the effect of different soil management practices on the microbial community of agricultural soil. The quality of agricultural soil is of vital importance to sustain our production of food and feed. The soil is a complex living ecosystem, soil organisms play crucial roles and provide many ecosystem services. Hence, biological parameters need to be developed and included to obtain better insights and understanding of soil health and functioning. These parameters need to be linked to practical management in such a way farmers can sustain or improve their soils. We aim to link soil functions carried out by microorganisms (fungi, bacteria and archaea) to soil management. To this end we apply 16s and 18s RNA sequencing to identify the microbial communities and use bioinformatics to predict whole metagenomes. One of the case studies compares the effect of liquid manure injection, which is common practice to mitigate ammonia emissions, and the application of solid manure. Preliminary results indicate that total diversity is lower in injected soils and show a decrease in Plant Growth Promoting Bacteria. Genes linked to plant beneficial traits such as ACC deaminase, are predicted in higher frequencies in soils that received solid manure. These preliminary results indicate differences in soil microbial communities caused by soil management are prevalent and possibly impact the functioning of the soil ecosystem.

P.75

THE EFFECTS OF ALGAE FERTILIZER ON WHEAT ROOT MORPHOLOGY ELUCIDATED USING PHENOTYPING AND METABOLOMICS

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One of the big challenges facing humanity is securing food and feed for future generations in a sustainable bioeconomy. The way fertilizer is used today aims at high yields without adequately considering the needs of plants and the preservation of our environment. The microalgae *Chlorella vulgaris* has been successfully used as a vector system to recycle phosphorus. In addition, our group found that *C. vulgaris* can fertilize wheat (*Triticum aestivum* L.) and modify the root architecture. The underlying mechanisms have not yet been investigated, and as a consequence, we hypothesize that: (1) nutrients from algae are available to wheat root uptake; (2) roots respond to algae fertilizer with changes in root architecture and morphology that are different to conventional fertilizer; and (3) active response of roots to algal nutrients is reflected in an alternate mode of nutrient uptake. We are analyzing single plants in highly controlled microenvironments that allow the direct

observation of morphological changes at the root micro- and macroscales. Simultaneously, we quantify changes in the phosphate pools released and transformed from algal components into the medium, their uptake from the medium, and their incorporation into the plant. These dynamics will allow the identification of the fertilizing phosphate components of the algal biomass. Their separation into different organic and inorganic fractions will allow the identification of the specific components available to root uptake. Effects of components on roots and the plant's nutrition will be assessed by metabolomics. A better understanding of the interface between algal nutrients and the root may enable future agricultural applications with sustainable use of algal biomass after it has been mined for other valuable compounds.

P.76

USE OF A MICRO-CAPILLARY SYSTEM TO EVALUATE NUTRIENT DYNAMICS IN RHIZOSPHERE SOIL SOLUTION OF MAIZE AND SOYBEAN

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Knowledge of rhizosphere processes is essential for characterizing soil nutrient availability. The objective of this study was to evaluate macronutrient dynamics in the rhizosphere of juvenile maize (*Zea mays* L.) and soybean (*Glycine max* L. Merr.). Plants were grown in soil collected from a long-term phosphorus (P) fertilizer trial, with and without tillage. Undisturbed blocks of soil (3x9x33 cm) were collected from plots that had received no P fertilizer and plots that had received 33 kg P ha⁻¹ y⁻¹ for 25 years. Soil blocks were placed in two-chamber mini-rhizotrons, maize seedlings were transplanted at the two-leaf stage, and grown for ten days. Soybean seeds were sown directly into the rhizotrons, and grown for 12 days. Micro-capillaries (1-mm diameter; 15 per chamber) were used to collect rhizosphere soil solution that is to be analyzed for pH, P, potassium (K), calcium (Ca), and magnesium (Mg), as well as phosphatase and organic acids (citrate, oxalate, malate). Samples are collected on a daily (24-h) basis. In preliminary research, maize shoot dry matter production was two-fold greater in P-fertilized soil (1.43 g plant⁻¹ versus 0.75 g plant⁻¹). Further, mean rhizosphere solution pH and P concentrations decreased with time in both amended and unamended soil. Within each treatment, P concentrations in rhizosphere solution were similar in surface versus subsurface soil layers, but were lower at the root surface than in bulk soil. When sample analysis is complete, results will allow us to evaluate the effects of long-term fertilizer P application on soil pH, nutrient cycling in the rhizosphere, and the response of maize and soybean roots to differing soil P supply.

P.77

BACTERIUM PAENIBACILLUS MUCILAGINOSUS ABI13 MAY REDUCE NUTRIENT LEACHING FROM AGRICULTURAL SOILS

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Improvement in nutrient use efficiency with concurrent reduction of nutrient losses from soils represent crucial steps for sustainable agriculture. In this regard, inoculation of novel bacterial strain (*Paenibacillus mucilaginosus* Abi13; MUCI) was tested in soil-plant microcosms with maize.

Concentration of nutrients in plant biomass as well as in soil solution were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Root exudation rates of low-molecular-mass organic anions (LMMOAs) were analysed by Ion Chromatography (IC) with conductivity detector. Changes in soil bacterial community structure were assessed by 16S rRNA gene sequencing.

Inoculation of MUCI led to significantly lower concentrations of cations (Ca, Mg, K) in soil solution while reducing the K uptake only. However, MUCI concurrently decreased plant-available NO₃⁻ concentrations inducing N deficiency in maize plants. The N deficiency consequently led to significantly lower exudation rates of carboxylates, mainly malate and citrate, by maize roots. Actinobacteria and Proteobacteria were the most affected phyla by MUCI inoculation in the maize rhizosphere.

The application of MUCI may represent promising strategy for reduction of nutrient leaching from soils, especially for divalent cations and nitrate. Further optimization of fertilization strategies complementary with MUCI inoculation is, however, needed in order to maintain biomass yield. Finally, our study demonstrated that inoculation of single bacterial strain into rhizosphere may trigger multiple reactions of the soil-plant system and may lead to significant changes in soil chemical and microbiological parameters as well as in overall plant nutrition.

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P.78

RHIZOSPHERE MICROBIAL COMMUNITIES AND DISTRIBUTION OF ASSIMILATED CARBON UNDER ZERO-TANNIN LENTIL GENOTYPES

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Root derived photosynthate is the primary source of readily available carbon (C) for rhizosphere microorganisms. These microorganisms provide precursors to stabilized soil organic matter by transforming plant root exudates into microbial biomass and accelerating the breakdown of plant tissues and root detritus. Tannins comprise a large group of plant phenolic secondary metabolites which alter rhizosphere soil microbial communities. Some annual legume crops have been selectively bred to remove tannins from their seed coats. Any shifts in the biotic processing of rhizodeposits from zero-tannin (ZT) genotypes have the potential to change

pulse crop-derived C trajectories through the soil. We used a ^{13}C pulse-labeling procedure to examine the incorporation of root derived C into individual phospholipid fatty acids (PLFAs) in rhizosphere soils of ZT and tannin (TAN) genotypes of lentil. Unconstrained ordination by non-metric multidimensional scaling of Bray–Curtis dissimilarity followed by permutational analysis of variance revealed significant differences in microbial community structure by tannin content but not genotype (tannin, $P=0.051$; genotype, $P=0.078$). Specifically, the relative abundance of Gram+ biomarkers (i15:0, a15:0, i16:0, i17:0 and a17:0) were more apparent under ZT genotypes while Gram– biomarkers (16:1 ω 9c, cy17:0, 18:1 ω 9,7,5c, cy19:0 ω 9,7c and 20:1 ω 9c) were more apparent under TAN genotypes. Moreover, the fungal to bacterial ratio was higher under ZT genotypes while the total microbial biomass was higher under TAN genotypes ($P=0.009$). Activities of the hydrolytic β -glucosidase and cellobiohydrolase and the oxidative phenol oxidase enzymes were assessed. Due to high variability, significance was not found, though generally, extracellular enzyme activities were higher under ZT genotypes (β -glucosidase, $P=0.045$; cellobiohydrolase, $P=0.062$; phenol oxidase, $P=0.182$). We have shown that differences in root derived C under ZT lentil genotypes, compared to the traditional TAN genotypes of lentil, directly affect microbial processing of these inputs. Consequently, ZT genotypes of lentil have the potential to alter C partitioning in the soil.

P.79 (will also be presented as poster pitch)

ROLE OF OPTIMAL ROOT MORPHOLOGY IN THE REDUCTION OF PHOSPHATE FERTILIZER APPLICATION IN OILSEED RAPE

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Oilseed rape (OSR, *Brassica napus* L.) is one of the most important oil crops in China, which needs more phosphorus (P) and shows more sensitive to phosphate (Pi) starvation than other crops. The application of Pi fertilizer is an important strategy to increase the seed yield of OSR. However, heavy fertilizer use cause environmental problems such as eutrophication of water sources, and there are limited natural resources of rock P. In this study, field trials were used to study the responses of five OSR cultivars under six Pi fertilizer application rate (P2O5) of 0, 15 kg/ha, 30 kg/ha, 45 kg/ha, 60 kg/ha (most farmers' fertilizer practices, FFP) and 75 kg/ha (P2O5, monoammonium phosphate). The responses of different cultivars to Pi fertilizer application was significantly different. Compared with cultivar Zhongshuang 11, cultivar Shengguang 168 increased the seed yield by 35% under 25% Pi reduction of FFP. The accumulation of P in shoot and seed of cultivar Shengguang 168 was significantly higher than that of Zhongshuang 11. Compared with Zhongshuang 11, Shengguang 168 had longer root length and root absorption area, and had a strong ability of P uptake and transport. Compared with Pi fertilizer application rate of 60 kg/ha, application of rooting agent under the treatment of 48 kg/ha (20% less Pi fertilizer than FFP) improved the root dry weight and increased the seed yield by 16.3% of Zhongshuang 11. Further results suggest that greater coarse root length in the surface soil (0-

10 cm soil layer) from the seedling to bolting stage enable more total P accumulation, shoot dry weight and seed yield in plants grown in soils with a low P supply.

P.80

RESTARTING RICE PRODUCTION AFTER DECONTAMINATION OF RADIOACTIVE CESIUM BY STRIPPING TOP SOIL AND SOIL DRESSING

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Aims and Background: Radionuclides were released from Fukushima Daiichi nuclear power plant because of the Great East Japan Earthquake disaster in March 2011. Radioactive cesiums (¹³⁴Cs and ¹³⁷Cs) are the major radionuclides deposited on the surface of the agricultural land. Several countermeasures have been implemented not to exceed limitation value (100 Bq/kg in the edible part of crops after 2012). The decontamination of radioactive Cs was the one of these measures by stripping the top soil of agricultural land and dressing non-contaminated soil. The aim of study was to confirm whether the agricultural land after the decontamination is available for safe rice production by assessing ¹³⁷Cs distribution in both soil and brown rice.

Methods: Rice cultivation was carried out in 2 sites of mountainous area in 2018, which is located in Kawamata town and Katsurao village. Working hours for plowing and ground leveling were measured. Also, plowing was practiced 2 ways which are conventional or careful. Careful plowing was deeper and slower than conventional that in order to mix well between subsoil and dressed soil. Rice growth condition was monitored and analyzed the its visible image of shoot by using drone. Rice yield was calculated by sampling 50 shoots. The variance of its yield was evaluated by harvesting each shoot of 18 spots in a field. Cesium-137 in soil was analyzed by NaI (TI) detector, ¹³⁷Cs in brown rice was measured by Ge detector.

Results: Compared with conventional plowing, careful plowing was decreased the variance of brown rice yield, and was taken time more. Rice yield was comparable with a target yield of Fukushima prefecture. Cesium-137 in brown rice was less than 1 Bq/kg.

Conclusion: After decontamination, rice production needed more time of plowing and ground leveling. Meanwhile, yield and quality of rice was preferable for farmer.

P.81 (will also be presented as poster pitch)

LONG-TERM AGRICULTURAL MANAGEMENT PRACTICES IMPACT DROUGHT RESISTANCE OF RHIZOSPHERE COMMUNITIES AND ACTIVITIES

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Increasing possibilities of severe droughts require the adoption of adaptive management practices that build up agricultural resilience in the face of climate change. However, knowledge gaps remain in understanding the impacts of these management practices on rhizosphere communities and processes, and complex ecosystem functions such as drought resistance. To understand responses of rhizosphere microbial community composition and activities to drought as affected by management practices, we conducted a field experiment with manipulated deficit irrigation at the Century Experiment at UC Davis after 25 years of contrasting agricultural management strategies. We hypothesized that the rhizosphere is plastic and that 1) communities will differ between conventionally and organically grown plants and 2) that differences in soil health properties and microbial communities between these systems will affect microbial resistance to drought. We quantified the interactions between soil moisture and long-term organic and conventional management practices on bacterial and fungal community compositions and functions, and activities of soil enzymes involved in C, N, and P cycling in the rhizosphere of tomato plants at various time points. We demonstrate that drought decreased the overall rhizosphere microbial diversity and altered microbial community composition. More importantly, management practices vary in their abilities to mitigate the loss of microbial diversity and maintain microbial community composition in response to drought in the rhizosphere. We also provide evidence that differences in resistant responses of microbial community are highly associated with soil pH and the activity of nutrient cycling enzymes. Our results suggest that sustainable management practices enhance the resistance of rhizosphere microbial community to drought and therefore provide untapped opportunities to build up agricultural resilience in the face of future climate change.

P.82 (will also be presented as poster pitch)

COMBINED EFFECTS OF PLANT WATER UPTAKE, N AVAILABILITY AND RHIZODEPOSITION ON DENITRIFICATION

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The main factors governing denitrification in agricultural soils are strongly influenced by growing plants. Soil moisture is dependent on root water uptake and plant transpiration. Plant N uptake largely controls N availability and competition for mineral N species among plants and microbes may have a strong influence on the soil microbial nitrifier and denitrifier community composition and microbial turnover rates. Nutrient availability further affects rhizodeposition and root decay, organic substrates that represent a major energy source for denitrifying microorganisms. We applied the ¹⁵N gas flux method to estimate N₂O and N₂ emissions of maize (*Zea mays* L.) with three fertilizer levels N0 / N1 / N2 (no / moderate / high N fertilization) and cup plant (*Silphium perfoliatum* L.) grown in a climate chamber. To link denitrification with available C in the rhizosphere, ¹³C labeling of plants was used to determine rhizodeposition. N availability strongly affected plant shoot and root growth, resulting in higher root/shoot ratio in N0 plants. Recovered ¹³C from rhizodeposition was highest in treatments with high N fertilizer level and high root dry matter. Soil moisture through differences in plant water uptake most strongly affected gaseous N emissions in our study. Daily N₂ fluxes were positively correlated with total CO₂ and ¹³CO₂ efflux from soil, especially in N0 treatments where N₂O+N₂ emissions were highest. Total N₂O+N₂ emissions were negatively correlated with plant ¹⁵N uptake and positively with soil NO₃⁻ concentration and N in microbial biomass. We conclude that plant water uptake is the most important regulator of denitrifying conditions in agricultural soils as it controls spatial distribution of water and air filled pore space. Under anaerobic conditions, however, small differences in C_{org} and NO₃⁻ availability largely affected gaseous N emissions.

P.83 (will also be presented as poster pitch)

DISSECTING RHIZOSPHERE RELATED TRAITS FOR PHOSPHATE EFFICIENCY IN EUROPEAN ELITE LINES AND LANDRACES UNDER LOW PHOSPHATE CONDITION

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During the last decades of maize breeding, little attention has been paid on performance on low phosphate conditions, as high fertilizer loads were used and the selection was performed under high phosphate conditions. Consequently, beneficial rhizosphere traits for high phosphate uptake efficiency may be lost in elite varieties.

The maize root has been extensively studied for rhizosphere and root architectural traits, especially that of tropical maize grown on low fertile, acidic soil. However, the performance of European modern elite genotypes on low phosphate soil with typically neutral or alkaline pH is unknown. This study aimed to fill the knowledge gap and identify the extent of variation in rhizosphere traits determining phosphate uptake efficiency using a germplasm pool, which covers modern elite lines, older material and recently developed double haploid progeny of landraces. Therefore, twenty-three inbred flint lines which were released in different ages (1950s-2010s) were grown at low phosphate level in local alkaline soil in artificial climate chambers. Rhizosphere and root parameters were observed in correlation to plant phosphorus acquisition. The results show that there is high variability among different lines of different ages, which may provide a new perspective for breeding.

P.84

ROOT-MICROBE INTERACTIONS IN MONOCULTURES AND WHEAT/LUPIN INTERCROPS

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Plant and soil types are usually considered as the two main drivers of rhizosphere microbial communities. The rhizosphere is an area of intensive interactions between plant roots and soil. In fact, the rhizosphere plays a key role by 'connecting' roots and the soil system, and regulating several aspects involved in plant nutrient cycling. The magnitude of rhizosphere effects varies widely among plant species and root biomass, possibly due to different quality and quantity of rhizodeposits. However, it is unknown whether or not rhizosphere effects are influenced by plant inter-species interactions, roots density or how these processes affect nutrient uptake by plants. This study of intercropping system was conducted to investigate the effects of rhizosphere-sharing of lupin, a phosphorus efficient plant on growth and nutrient uptake of wheat under different seed density 150, 200 and 250 kg ha⁻¹.

Five months after sowing soil available nitrogen did not differ significantly in intercropping, but did increase in lupin monoculture (particularly for nitrate). Intercropping significantly reduced available phosphorous. In the majority of soil enzyme activities did not differ between monoculture and intercrops, with the exception of soil acid phosphate activity. We found that the wheat/lupin cropping system did not impacted soil basal respiration and microbial biomass.

These field experiments gave results for understanding the biological interactions between plants (wheat/lupin) and soil enzyme activities, while it would also provide valuable information regarding crops productivity and nutrient uptake.

P.85

INTERSPECIFIC DIFFERENCES IN PLANT GROWTH PROMOTING BACTERIA - A GENOMIC ASSESSMENT

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Both bacteria and fungi are proving to be valuable plant stimulants for agricultural practices and of increasing interest for commercialization. The challenge of finding efficient microbes to associate with plants is to detect reproducible compatibilities between plant species and microorganisms. It is necessary that the benefits of microbial associates be reproducible and consistent. A successful bioproduct output includes a deep assessment of microbial genomic features coupled with large plant-bacteria screening in variable environmental conditions. We tested the inoculation of 220 bacterial endophytes in Arabidopsis plants growing in MS medium, narrowing down to a selection of 5 isolates that consistently promoted plant growth, including two strains of *Plantibacter flavus* as the top plant growth promoters. These select isolates were inoculated to basil, bok-choy, and lettuce plants growing under aquaponic system and clearly showing plant growth-promoting effects: *P. flavus* strain M251 promoted the greatest beneficial effects for Arabidopsis growth and improved the roots biomass for lettuce and basil, while the inoculation with *P. flavus* strain M259 significantly increased aerial biomass in Arabidopsis and bok-choy, but not in lettuce and basil. Whole genome comparison was performed to assess the differences between the two *P. flavus* strains, and differential potential to produce different small molecules, secondary metabolites, and enzymes between the two strains were taken as the main cause for the observed interspecific variance in the plant-microbe associations. Bacterial endophytes can release a myriad of molecules that varies at strain level and a successful association will depend on how they both plant and bacteria complement each other in exchanging different molecules that may be useful for both sides in similar metabolic pathways.

P.86 (will also be presented as poster pitch)

EFFECTS OF NO₃⁻:NH₄⁺ RATIOS ON GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF N-EFFICIENT AND N-INEFFICIENT RAPE (*BRASSICA NAPUS* L.) SEEDLINGS

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At present, there is a problem of excessive use of single nitrogen fertilizer and low utilization rate in rapeseed production. A high concentration of ammonium (NH₄⁺) as the sole source of nitrogen in the growth medium is often toxic to plants, especially in the *Bassica napus* which is an important oilseed crop in China. Therefore, N-efficient (D4) and N-inefficient (D2) materials were irrigated with a nutrient solution containing different NH₄⁺: NO₃⁻ ratio (100:0, 75:25, 50:50, 25:75, 0:100 total 6 mmol/L N). Results showed that there was a significant difference in the growth of D4 and D2 under different ratios of nitrogen sources. Low-efficiency varieties have a higher plant growth-related parameters in NH₄⁺: NO₃⁻ ratio (75:25) compare to the other three treatments, which exhibited a higher root morphology, larger biomass, meanwhile, it was showed higher nutrients accumulated, and predominant nitrogen use efficiency in NH₄⁺: NO₃⁻ ratio (75:25), resulting in a decrease of

malondialdehyde (MDA) content in roots. However, N-efficient (D4) showed a better growth characteristic under $\text{NH}_4^+:\text{NO}_3^-$ ratio (0:100) compared to the corresponding treatments. Moreover, $\text{NH}_4^+:\text{NO}_3^-$ ratio (0:100)-induced the lower accumulate of MDA by reducing the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities in roots of N-efficient seedlings. Furthermore, the N contents in the old leaves were enhanced with the increase of the ratio of $\text{NH}_4^+:\text{NO}_3^-$ in D4, while reduced the N content in the new leaves, there was no obviously difference was observed in roots under the difference ratio of $\text{NH}_4^+:\text{NO}_3^-$. Overall, our results suggest that low-efficiency varieties prefer the growth environment of $\text{NH}_4^+:\text{NO}_3^-$ ratio of 75:25 while high-efficiency materials are more suitable for pure nitrate nitrogen environment (the ratio of $\text{NH}_4^+:\text{NO}_3^-$ (0:100)).

P.87

DIFFERENCES OF UNAVAILABLE PHOSPHORUS UTILIZATION ABILITY IN JAPANESE WHEAT VARIETIES

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A large amount of soil phosphorus (P) is unavailable for plant. Enhancing P efficiency in agriculture can be achieved through improving P acquisition from unavailable P. In Japan, agricultural soils are typically enriched in P as a result of long-term, excessive P fertilization. However, direct investigation about P fractions utilized in the rhizosphere is limited. In this study, we aimed to evaluate the differences in unavailable P utilization among Japanese wheat varieties.

Nineteen six varieties of wheat (*Triticum aestivum* L.) plants were grown in soil pot culture. Treated soils with P fertilization (HP: 200 mg-P kg^{-1}) and without (LP) were established using low-P field soil of Hokkaido University. Seedlings germinated in dishes were transplanted to a pot filled with 300 g soil. Plants were grown in a greenhouse until the ripening period from May to December. The plant tissue was harvested separately (i.e. shoot, panicle, and root). Dry weight and P content were measured. Rhizosphere soils of LP were collected and P fraction was measured by Hedley method. Based on the dry weight of the shoot, root, and seed, low-P tolerance value of individual wheat variety was calculated.

As a result, we found distinctive differences in low-P tolerance value among the wheat varieties. Interestingly, the availability of P fractions, such as $\text{NaHCO}_3\text{-Po}$, NaOH-Pi , and Po was also different among the varieties. Moreover, among the plants which showed the same low-P tolerances value, the amount of unavailable P in the rhizosphere was different. This result indicates that the kinds of wheat have a different mechanism of P acquisition from the soil. These findings have important implications for improving P acquisition from unavailable P in the soil of wheat.

P.88

OPTIMIZING ASSAY PH VALUES FOR ENZYME ACTIVITIES ACROSS A SOIL PH

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Extracellular enzyme assays are commonly used as indicators of biochemical functions in bulk and rhizosphere soils. Although the majority of enzyme assays are conducted at a standardized pH (e.g. pH 5.0), the pH optimum in soils can vary greatly and result in inaccurate conclusions. The objective of this study was to compare optimum pH for enzymes across a range of soil pH. In 2017, soils (0-15 cm) were collected from a heathland restoration project (est. 1999) on the Isle of Purbeck, UK with treatments of elemental sulphur (ES) or ferrous sulphate (FS) compared to a control, acid grassland (AG) and heathland. Differences in soil pH were still evident in 2017 with the control (pH 5.8) and FS (pH 5.6) significantly higher than ES (pH 4.6), AG (pH 4.8) and heathland (pH 4.4). Enzyme assays used fluorescent substrates for b-1,4-glucosidase (b-gluc), B-N-acetylglucosaminidase (NAG) and phosphatase with modified universal buffer (MUB) adjusted to pH 3, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, 10, and 12. The optimum pH for enzyme assays varied from pH 3-4.5 for b-gluc, pH 4-5 for NAG and pH 4-6 for phosphatase. Comparisons at individual MUB pH resulted in different conclusions compared to optimum pH. For example, b-gluc activity at pH 5 for the control was significantly higher than ES, AG, and heathland. However, the AG (48.38 $\mu\text{mol MU}^{-1}\text{g}^{-1}\text{hr}^{-1}$) and heathland (47.07 $\mu\text{mol MU}^{-1}\text{g}^{-1}\text{hr}^{-1}$) were significantly higher when pH optimums were considered at pH 3, compared to pH 4.5 for the control (27.99). Phosphatase activity at MUB pH 5 indicated no difference among the treatments despite the heathland soils resulting in 86.07 $\mu\text{mol MU}^{-1}\text{g}^{-1}\text{hr}^{-1}$, compared to 41.01, 25.39, 39.17 and 47.63 $\mu\text{mol MU}^{-1}\text{g}^{-1}\text{hr}^{-1}$ for the control, FS and AG treatments, respectively at optimum pH. The standard assay underestimated function in low pH soil, demonstrating the importance of optimum pH for enzyme assays, especially when comparing diverse soils.

P.89

RHIZOSPHERE PROCESSES INFLUENCING PHOSPHORUS CYCLING IN SOIL

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Amount and type of rhizodeposit can influence phosphorus (P) availability directly by desorption or dissolution or indirectly by promoting increased microbial activity that leads to the decomposition of soil organic matter. In this study we aimed to determine how low P availability influences plant root exudate-induced, microbe-mediated decomposition of soil organic matter (i.e., rhizosphere priming), and understand physical and biogeochemical processes affecting the transformation, movement, and storage of P (specifically organic P). Rhizoboxes were filled with soils and root exudate compounds glucose, alanine, oxalate, and their mixture were introduced into the soil for 15 days delivered through an artificial root. Zymography and planar optodes were used to image acid phosphatase activity, and O₂ and pH distribution, respectively. Soil P species were

determined using sequential extraction, ^{31}P solution NMR, and ultra-high-resolution mass spectrometry. Root exudates had a distinct influence on the distribution of soil pH and O_2 concentration, and distribution and concentration of potential acid phosphatase activity. Among the exudate compounds, resin extractable P_i , dissolved organic carbon, and water extractable Fe, and Al were greatest in oxalate treatment. These results suggest destabilization and release of mineral associated organic matter. ^{31}P solution NMR analysis of soils from the rhizosphere show distinct changes in P_o monoester to P_i ratios that are indicative of exudate specific decomposition of P_o . Further, both ^{31}P solution NMR and ultra-high-resolution mass spectrometry analysis show an absence of P_o compounds in oxalate treatment suggesting P_o mineralization. Total microbial biomass was lowest in the oxalate treatment and the community structure in the rhizosphere soil of oxalate treatment was distinctly different than other treatments. These results show the influence of root exudate composition on mineral destabilization and release of MOAM and its potentially important contribution to phosphorus cycling.

P.90

SOIL MICROBIAL COMMUNITY STRUCTURE IS SHAPED BY LONG-TERM FERTILIZATION REGIMES OF CORN CROPS

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The intensification of agricultural systems is generally accompanied by increasing nitrogen fertilization to improve crop yields. However, excess nitrogen, if not lost from the soil system, could alter the structure and functionality of soil microorganisms, potentially affecting soil health. Since such effects could be enhanced by repeated application of nitrogen fertilizers, the aim of this study was to characterize the long-term effect of nitrogen fertilization rates on soil microbial communities. The study was carried out in a 10-year experiment located in Elora, Ontario, where corn (*Zea mays* L.) crops were continuously treated with different pre-plant N-fertilization rates: 0, 28, 57, 115, 188 and 230 kg N ha⁻¹. In each plot, composite samples (0-15 cm) were collected within 10 cm of the crop (reproductive stage) and stored at -20°C until DNA was extracted. Amplicon-based sequencing of fungi (ITS1), bacteria and archaea (16S rRNA V4) was carried out using Illumina MiSeq, and sequences were analyzed with QIIME2. Even though long-term nitrogen rates did not affect alpha diversity in the targeted groups, they were affected in terms of community composition (Bray-Curtis and weighted-UniFrac dissimilarity measures). Still, microbial composition presented high spatial variability within treatments, and differences were clear mostly between the lowest and highest nitrogen rates. Additionally, soils with the highest nitrogen rate tended to have lower proportion of arbuscular mycorrhizal fungi and higher proportion of archaea. Since microbial communities were relatively homogeneous, we argue that the strongest effect of nitrogen levels occurs following fertilization. In the long term, other factors (e.g., edaphic properties, crop species, management) also seem to have a strong influence in shaping these microbial communities. Because microbial functionality could be substantially altered by nitrogen inputs, we will also inquire in the putative function of these groups using bioinformatics tools.

P.91

THE BIODIVERSITY OF EUKARYOTES IN BAMBARA GROUNDNUT RHIZOSPHERE

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Bambara groundnut has been observed to resist pest and drought, and still able to produce enormous yield when cultivated on poor soil. The advantages of the crop to farmers includes the fact that it produces enormous yield with very low agricultural input. The aim of the present study was to determine the taxonomic and microbial diversity, and identification of eukaryotic organisms in Bambara groundnut rhizosphere using microbiome analyst platform.

A total of ten soil samples corresponding to the different growth stages were collected from Bambara groundnut rhizosphere over interval period of 4 weeks at North-West University agricultural farm, Mafikeng campus. These samples were assessed for the presence of eukaryotic organisms through polymerase chain reaction (PCR) of 16S ribosomal ribonucleic acid (rRNA). Metagenomics analysis using culture-independent technique (next generation sequencing (NGS)) by Paired end illumina-Miseq™ technology sequencing with the prospect of discovering novel eukaryotes with plant growth promoting features was used. Statistical analysis were used to profile and confirm identities of detected organisms.

Fifty nine (59) features were detected from the 10 samples by microbiome analyst under data normalization and data cleaning. Taxonomic analysis showed that, 69% of the eukaryotes in the samples were *Peronosporales* while *Thalassiosiraceae* and others were 30% and 1% respectively.

There was profound variance difference in the rhizosphere microbiome mainly at the OTU level which largely attributed to those taxa most strongly depleted by the plant. *Thalassiosira pseudonana*, which is a centric diatom found in marine environment was observed in this study. This is the first time so far that *T. pseudonana* is observed in plants' rhizosphere and its ability to withstand harsh environmental variation might contribute to the ability of Bambara groundnut to be able to withstand drought, pests and diseases.

P.92

ARBUSCULAR MYCORRHIZAL FUNGAL CORE MICROBIOME, DIVERSITY AND COMMUNITY STRUCTURE IN ROOTS AND RHIZOSPHERE OF WHEAT, PEA AND CANOLA ARE INFLUENCED BY THE ROTATION SYSTEM

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With \$26.7 billion injected into the economy each year, canola is the most profitable crop in Canada. Canola is a non-mycotrophic plant that does not actively associate with arbuscular mycorrhizal fungi (AMF). In western Canada, Canola is grown in rotation with other crops, being most often cereals and pulses. It is recognized that AMF allow mycotrophic crops to access a larger pool of nutrients and increase plant health and productivity.

However, the widespread use of canola in rotations raises concerns on the loss of soil microbial diversity and increased susceptibility of crops to pathogens. We hypothesized that crop rotations with higher frequency of mycotrophic plants could alter the root and rhizospheric microbiome of the crops present in the rotation and increase the overall productivity of the production system. However, the effect of the frequency of each rotation-species on the microbiome and productivity of each crop are still poorly understood. The main objective of this research is to characterize the AMF community of wheat, pea and canola under different rotation systems in order to understand the effects of crop frequencies on the root and rhizospheric microbiome of each crop and on plant productivity. To do this, three rotation systems (intensifying canola, cereals or pulses over four years) were tested in a complete random blocks design. The root and rhizosphere microbiomes were sampled for each of the rotation phases at two growing stages. The rotation system and the growth stages had an impact on the biodiversity and structure of AMF communities in all crops. However, only wheat has seen its productivity increased in the pulse-intensified rotation. We were also able to link the relative abundance of specific AMF species to higher productivity of wheat, pea and canola.

P.93

PGPR STRAINS FROM THE RHIZOSPHERE OF A XEROHALOPHYTIC PLANT PROMOTED HEMEROPHYTIC PLANT GROWTH, FROM MODEL PLANTS TO FORAGE PLANTS

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Plants grows in arid regions play key roles in desert control. The xerohalophyte *Haloxylon ammodendron*, called “desert forest”, has a pivotal position in fighting against desertification in the northwestern China. With the attention to the safety of agricultural production and seeking effective substitute for chemical fertilizer, scientists focus on plant growth promotion rhizobacteria (PGPR). In this paper, we analyzed and illustrated phylogenetic diversity of culturable rhizosphere bacteria of *H. ammodendron* with different growth years in Alxa Youqi County, Inner Mongolia, in Badain Jaran Desert and Minqin County, Gansu Province, in the Tengger Desert, respectively, by 16S rRNA gene sequence and identified candidate strains of novel species by polyphasic taxonomy. Moreover, screening dominative strains with stress tolerance, and verifying their functions in enhancing plant resistance and promoting plant growth. The main results are as follows: 1. The bacterial community composition and the preponderant colony in two deserts are nearly same. There are unique strains in the two regions. 2. We isolated 293 culturable bacterial strains that belonged to six phylogenetic groups: Actinobacteria, Firmicutes, Bacteroidetes, α -Proteobacteria, γ -Proteobacteria and β -Proteobacteria. 3. We obtained 36 strains of potential novel bacterial species, some with PGPR characteristics like strain WM13-24 belonging to the genus *Bacillus* and M30-35 to *Pseudomonas*. At present, we conducted interaction experiments with the two strains, respectively, with *Arabidopsis thaliana*, *Medicago truncatula*, ryegrass, alfalfa and white clover. The results showed that the two strains had fine growth-promotion effects on those plant species, and further studies are in progress. In conclusion, we clarified diversity of rhizosphere bacteria, isolated

unique strains and provided bacterial resources from the rhizosphere of *H. ammodendron* under high saline and drought conditions. The strains with PGPR characteristics enriched PGPR resources for developing new type of microbial fertilizers with stress resistance and growth-promotion effects on *H. ammodendron* itself and hemerophytes as well.

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P.94

MANIPULATING THE ROOT MICROBIOME WITH ORGANIC ANION EFFLUX FROM WHEAT ROOTS

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As plant roots grow through soil they inevitably interact with a plethora of microorganisms. Some microbes are attracted to, or repelled by, the chemical changes that occur in the rhizosphere and so the microbiome in the rhizosphere are usually very different from the bulk soil. The microorganisms closely associated with root tissues (in the tissues or on the surface) affect plant growth most. This provides an opportunity for increasing crop productivity more sustainably if we are able to manipulate the composition of the root microbiome in a beneficial way. To achieve this we first need to know how to define the microbiome more accurately and how to manipulate it in a predictable manner. In this study, we tested whether the microbiome on wheat roots is affected by the release of simple organic anions. A pair of near-isogenic wheat lines that vary in citrate efflux (*Citrate-line* and *Null-line*) were grown in three different soils and the bacterial and fungal communities at different positions along the length of the seminal and nodal roots were analysed. We found that the microbiome was affected by soil type (acidic or non-acidic), root type (seminal or nodal) and position on the root (tip or base). Citrate efflux from the root tips also affected the microbiome composition in acidic and non-acidic soils but the effect was smaller than for the other variables. These results indicate that it is misleading to describe “the root microbiome” on a particular plant because it clearly varies with the environment, root type and with location on the root. We also demonstrate that the microbiome can be altered by the release of a simple organic compound. This information helps us understand how to engineer the microbiome in a reliable fashion to develop more sustainable production systems.

P.95 (will also be presented as poster pitch)

CIRCADIAN RHYTHM OF MICROBIOME IN RICE RHIZOSPHERE

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Circadian clock is a critical molecular mechanism in organisms to respond to daily cycles of light and dark and/or other environmental factors. Recently findings reveal that rhizosphere microbiome was able to adapt the change of circadian cycles of the rhizosphere environment. Yet, the underlying mechanisms of the circadian rhythm for rhizosphere microbiomes are still unclear. Hence, we investigated circadian rhythm of rhizosphere microbiome in rice rhizosphere, distinguished the endogenous rhythm microbial group from exogenous ones, and built a bridge between circadian rhythm of rhizosphere microbiomes and biogeochemical processes in rhizosphere. Results from this work will bring us knowledges of the impacts of circadian rhythm of microbial communities on ecological processes in rhizosphere. As a new perspective, it may be a key point in truly understanding the cross-talking between roots and microorganisms. Therefore, this work may lead us to a new cognition of rhizosphere microbial ecology.

P.96

IMPACTS OF CLIMATE CHANGE ON STRUCTURE AND FUNCTIONING OF WHEAT AND BARLEY RHIZOBIOMES IN CONVENTIONAL AND ORGANIC FARMING

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Soil microbes drive important processes in agroecosystems such as degradation of organic matter and nutrient release from organic and mineral compounds. However, facing land use intensification and climate change, soil microbial communities are subjected to increasing environmental pressure. We investigated the climate change impacts on the structure and functioning of wheat and barley rhizobiomes in a conventional and an organic farming system in the “Global Change Experimental Facility”. Bacterial rhizosphere communities were analyzed at vegetative and generative cereal growth stages by 16S Illumina amplicon sequencing. Activities of hydrolytic enzymes involved in soil C, N and P cycling were estimated by community-based prediction tools (PanFP, Tax4Fun) and compared with measured activities using fluorescently labelled substrates.

The microbial community structure was mainly affected by crop species and soil pH, while agricultural management showed minor effects. In contrast, a strong impact of agricultural management on enzyme activities was detected. Consequently, we revealed poor concordance between measured and predicted enzyme activity potentials, indicating a limited informative value of these tools. Climate change effects were neither detected for the structure nor for the functions of the bacterial communities. These findings were supported by a selective cultivation-based approach focusing on the P-solubilizing rhizosphere community of wheat, which was dominated by the genera *Phyllobacterium*, *Pseudomonas* and *Streptomyces*. We found

impacts of the wheat development stage and of the agricultural management on the abundance, diversity and functions of P-solubilizing bacteria, but climate treatments showed only marginal effects.

Our data indicate that structure and activity of the rhizosphere community can be steered by agricultural management options, such as crop selection, liming and fertilization. Moreover, the moderate, but realistic climate manipulations in our experiment indicate that the rhizosphere communities are resistant against climate change, suggesting their high potential to buffer negative impacts of climate change on agroecosystem processes.

P.97

IMPACTS OF SOIL BORNE DISEASE ON PLANT YIELD AND FARM PROFIT IN DAIRYING SOILS OF NEW ZEALAND.

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The extent and cost of soil disease constraints in high-value dairy systems in New Zealand is currently poorly understood for the major pasture plants used in these systems. Regional measures of disease pressure were obtained by comparing white clover, perennial ryegrass and plantain growth in 30 farm soils with (non-pasteurised) or without (microwave pasteurised) their normal complement of microbial and nematode pathogens. Pasteurising soils from the Waikato (North Island) region led to significant increases in clover (35%) and ryegrass (19%) shoot dry matter. Individual site yields increased up to 74% for clover and 38% for ryegrass. For Canterbury and Southland (both South Island), there was no increase in plant yield with pasteurisation regionally, but increases in either clover or ryegrass were significant at three individual farm sites.

Of the abiotic factors associated with soil disease across all regions, negative linear relationships between disease pressure (% growth change) and anaerobically mineralisable nitrogen:total nitrogen ratio (an indirect measure of microbial activity) were found for clover and ryegrass, accounting for 34% of the variation in plant growth for both.

A positive linear relationship was found between disease pressure and *Heterodera* cyst nematode abundance, accounting for 33% and 17% of the variation in clover and ryegrass growth, respectively. Illumina Mi-seq was used to elucidate the rhizosphere bacterial and fungal communities associated with reduced clover and/or ryegrass yield in non-pasteurised soil. Culture-dependant methods were used to isolate pathogenic microbes from root endospheres. It is clear that a complex of soil-borne root pathogens is involved in plant growth reduction in New Zealand pasture soils.

Our results suggest that these root pathogens are most prevalent and damaging in the Waikato where economic modelling estimated clover and ryegrass root disease to cost 211 kg MS ha⁻¹ year⁻¹ in milk production and \$NZ909 ha⁻¹ year⁻¹ in farm profitability.

P.98

LONG-TERM NUTRIENT ADDITION MEDIATES PLANT-MICROBIAL ASSOCIATIONS BY ALTERING MICROBIAL METABOLISM

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Soil microorganisms in low-nutrient ecosystems promote plant growth by enhancing nitrogen and phosphorus acquisition from soil; and in exchange, plants provide carbon to the microbial community. Beneficial plant-microbe associations that facilitate nutrient acquisition are sensitive to fertilization effects, which can alter soil microbial community composition and function. Over the last century, increased fertilizer use and burning of fossil fuels have increased global nitrogen and phosphorus deposition rates leading to nutrient enrichment in low-nutrient ecosystems. Our objective is to characterize changes in bulk and rhizosphere soil microbial community composition and function in a wetland experiencing nutrient enrichment. We hypothesize shifts in microbial community composition due to long-term nutrient additions are reflective of differences in microbial metabolism. To examine long-term fertilization effects on plant-microbial interactions, we used a long-term (since 2002) factorial experiment to test effects of nutrient addition (N-P-K fertilizer) and disturbance (mowing) on microbial diversity and function. We sampled bulk soil and rhizosphere soil from two plant species in eight mowed plots half of which received fertilizer. Bacterial community composition differed between fertilization treatment and bulk and rhizosphere soils (PERMANOVA $R^2=0.31$, $p<0.001$; $R^2=0.22$, $p<0.001$). Additionally, there was a higher ratio of copiotrophic to oligotrophic bacterial taxa in nutrient enriched plots, which is indicative of metabolic changes within the microbial community. To investigate effects of fertilization on microbial metabolism, we used a metabolic fingerprint approach using Biolog carbon, nitrogen, and phosphorus microarrays to measure changes in microbial substrate use rates and diversity. Preliminary microarray results suggest that microbial communities from nutrient enriched plots metabolize a greater diversity of substrates faster than in ambient plots. Thus, long-term fertilization and disturbance altered taxonomic and metabolic diversity of bulk soil and rhizosphere microbial communities. These results in combination with targeted plant growth assays will inform how changes in microbial community metabolism impact plant growth.

P.99

IDENTIFYING CULTIVAR INFLUENCES ON THE OILSEED RAPE MICROBIOME

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Oilseed Rape (*Brassica napus*), is the second most important Oilseed crop in the world with 70million tonnes produced globally in 2017. Over the past five decades global trends have shown increased cultivation with an associated increase in overall yields. Within the United Kingdom, a plateau in overall yield has been observed despite increased cultivation, with research indicating a link to the development of an 'unhealthy' rhizosphere microbiome. Plant genotype (cultivar) has been shown to influence the structure of rhizosphere microbiomes across plant species with potential applications in the engineering of 'healthy' microbiomes. Given the potential

applications of cultivars, this work sought to elucidate the influence of cultivars on the root and rhizosphere microbiomes of Oilseed Rape in agriculturally relevant conditions. Four Oilseed Rape cultivars representing extremes of root biomass (Canard & Catana) and plant metabolite exudation profiles (Compass & Temple) were used. Twenty-one replicate plots of each cultivar were maintained for one growing season in a UK field under standard agronomic practices. At the end of the growth season, samples of root tissue and rhizosphere soil were collected for analysis of bacterial, fungal and protist communities by amplicon sequencing. Downstream analyses included assessment of microbiome structure, diversity and analysis of inter kingdom networks. Significant differences in the structure of bacterial, fungal and protist communities were identified between root and rhizosphere microbiomes across all cultivars. However, fewer significant cultivar-based differences were observed, with similarity analysis identifying dissimilarities within bacterial and protist rhizosphere communities in certain cultivars. Together these analyses suggest, that under agriculturally relevant conditions, cultivar influences upon the root and rhizosphere microbiome of Oilseed Rape are minimal, with differences occurring due to small shifts over many taxa.

P.100 (will also be presented as poster pitch)

THE RHIZOSPHERE METAGENOMES OF ANCESTRAL AND MODERN WHEAT CULTIVARS GROWN UNDER LOW FERTILISER INPUTS ARE NOT SIGNIFICANTLY DIFFERENT

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With the rock phosphate reserves approaching depletion and the increasing concerns about the environmental and economic costs of fertilizer use in agriculture, there is mounting pressure to reduce their intensive use. However, recent breeding efforts were mostly carried out under high levels of fertilizers, which has led to the hypothesis that modern wheat cultivars might have lost their capacity to associate with soil microbes that would help them acquire nutrients from the soil under low fertilization conditions. To test this hypothesis, ten ancestral and modern wheat cultivars belonging to two wheat species (*Triticum aestivum*-hexaploid, *Triticum turgidum*-tetraploid) were seeded in a field experiment under low fertilization conditions. At the post-anthesis stage, the rhizosphere soil was collected, its DNA extracted and sent for shotgun metagenomic sequencing. In contrast to our hypothesis, there was no significant difference in the rhizosphere metagenomes of the different cultivars, and this held true when focusing the analyses on specific taxonomic or functional categories of genes. The main factor influencing the rhizosphere metagenome was space, with samples collected from different parts of the field showing significant differences in their genetic and taxonomic content. This variability was also visible for the yields and other agronomic parameters measured at the end of the growing season, which results in significant correlations between these parameters and N and P cycling genes, which would need to be further scrutinized to ascertain their roles in wheat nutrition and growth. Our study showed for the first time that the rhizosphere metagenome of wheat is stable across a wide variety of genotypes, which suggests that modern genotypes did not lose the ability to associate with beneficial nutrient-cycling soil microbes.

P.101 (will also be presented as poster pitch)

DEVELOPMENTAL PROGRAMME AND ITS INTERACTION WITH RHIZOSPHERE MICROBE IN MAIZE

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The microbiome colonizing the rhizosphere (soil close to root surface) determines the root-microbe interaction of their hosts by balancing the growth and immune response. Developmental complexity of single root challenges detailed studies to explore the mechanism underlying the functional dynamics of host root growth and spatial assemblages of microbiomes. Our research focuses on three distinguished root zones (elongation zone without root hairs and lateral roots, differentiation zone with root hairs and lateral root primordia, and maturation zone with emerged lateral roots) with divergent biological statuses along the longitudinal axis of *Zea mays* (maize) root. By a large-scale field study of 20 genetically distinct maize inbred lines, transcriptomic dynamics by RNA sequencing (RNA-seq) and rhizosphere microbiota by 16S/ITS amplicon sequencing indicate the functional and physical interaction between host root and environmental microbes. Integration of gene co-expression analysis of root transcriptome and co-occurrence analysis of rhizosphere microbiome provide the hints that lateral root formation is the important internal trigger to drive microbiota differentiation at the root-soil interface, which is also validated by diverse monogenic mutants of maize *rum1*, *lrt1*, *rtcs* and *rth6* with developmental defects of lateral roots or root hairs. Lateral root initiation is also necessary for sustaining arbuscular mycorrhizal (AM) colonization for the host. To testify how AM fungi interacts with lateral root initiation, systemic investigation of pericycle (initials of lateral root formation) cell-type RNA-seq based on laser capture microdissection (LCM) together with tissue-specific metabolome and proteome analyses of lateral root defective mutant *lrt1* of maize, we identified a novel regulator controlling AM fungal-induced pericycle competence during lateral root initiation. This mechanism is validated by a recombinant inbred lines population as well. Taken together, our results will be useful and promising to understand the plant-microbe interaction and to guide the future crops breeding with growth promotion and disease suppression.

P.102 (will also be presented as poster pitch)

MICROBIOME-GUIDED SELECTION TO IDENTIFY PLANT GROWTH-PROMOTING BACTERIA THAT ENHANCE JUVENILE MAIZE GROWTH UNDER COLD STRESS

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Maize is the third most important staple crop worldwide, however, due to its subtropical origin, maize growth in the northern hemisphere has brought up some challenges. Low temperatures are posing stress on the plant growth, resulting in eventual yield losses. One of the proposed ecological solutions to boost crop yield are the use of plant-growth promoting rhizobacteria. To facilitate the identification of new bacterial strains that promote plant growth, we took a microbiome-guided approach. The maize root endosphere microbiome was studied in depth based on five different experiments. At first, the main maize root endosphere families were identified based on two independent experiments for which maize was either grown in the field or in pots filled with field soil from the same field. We detected twelve families that were repeatedly enriched in the endosphere and highly abundant and identified these as the main root endosphere families. Further on, to detect the contribution of seed endophytes in the root endosphere, *in vitro* grown and soil-grown endospheres were compared, revealing the small importance of seed endophytes in the root microbial community of two-week old maize plants grown in soil. Moreover, the effect of cold, having a major impact on maize growth, was investigated on the maize microbial communities. In two independent experiments, the bacterial families that change upon cold stress were detected resulting in a list of 12 bacterial families that shift upon cold. Further, a bacterial collection of maize endophytes was established of which a subselection was screened for growth promotion of maize under cold temperatures. This screening resulted in the identification of two promising growth-promoting strains for maize.

P.103

ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY RESPONSE TO FERTILISER AND CROP ROTATION IN WHEAT RHIZOSPHERES OVER MULTIPLE YEARS

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Introduction: Arbuscular mycorrhizal fungi (AMF) are soil microbes that colonize up to 90% of all land plants and offer benefits in the form of nutrient acquisition, water retention, and resistance to pathogens and pests. AMF diversity is thought to respond to a range of biotic and abiotic factors in soil management practices.

Methods: The influence of management on AMF diversity was studied in a multi-year wheat field trial incorporating variation in fertiliser application (N and P) and crop rotation. AMF diversity in root-associated soil was analysed by PCR amplification with AMF-specific DNA primers that span the small sub-unit to the large sub-unit of the ribosomal RNA gene and the products were sequenced using Illumina MiSeq. These primers were chosen for this study because they provide greater coverage and depth of AMF sequences compared to traditional fungal primers.

Results: The AMF diversity in individual samples differed by treatment in fertiliser type and amount and previous crop rotations of canola or chickpea. Bioinformatic analyses showed a strong correlation in particular between genotype diversity within the AMF genus *Funneliformis* and rhizosphere samples with no fertiliser treatments, and those grown in conjunction with chickpea during rotation years.

Conclusions: The results indicate that management treatments have a strong influence on AMF species diversity in agricultural trials with varying inputs of nitrogen and phosphorus, and differing crop rotations. These differences may be explained by the filling of niches dictated by functional traits at the AMF genus or species level. Further research on functional traits will elucidate how AMF can be strategically applied for reducing chemical inputs and water use in agriculture.

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CORRELATION OF THE BACTERIAL MICROBIOME, GENOTYPIC VARIANCE AND ALKANNIN/SHIKONIN CONTENT OF *ECHIMUM VULGARE* L., A PLANT WITH POTENTIAL MEDICINAL PROPERTIES

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Several members of the plant family Boraginaceae are confirmed producers of the secondary metabolites alkannin and shikonin. These compounds bear several biological activities such as anti-inflammatory or antibacterial effects or acceleration of wound-healing. *Echium vulgare* L., a common Boraginaceae species native to Europe, produces alkannins, which are mainly found in the periderm of *E. vulgare* roots. As microorganisms have been reported to interact with secondary metabolism of some plants our aim was to see whether there is a potential link between plant microbiota and secondary metabolite production in *E. vulgare*.

We collected plants at two different growth stages of wild *E. vulgare* at seven different sites in Austria to correlate bacterial community patterns, genotypic variance and the production of secondary metabolites. We analysed microbial community composition in different sections of the root system, in the surrounding rhizosphere and bulk soil by next generation sequencing of 16S rRNA genes. Furthermore, we genotyped 64 individuals of *E. vulgare* using 12 microsatellite markers and determined total alkannin/shikonin content of dried root samples by ultra-high-performance liquid chromatography-high-resolution mass spectrometry. The high variance of alkannin/shikonin content in the collected *E. vulgare* roots in our study suggests that factors like the genotypic variance or associated microbiota may influence secondary metabolite production. According to our results we will discuss how microbiota composition and plant population genetic differences correlate with alkannin/shikonin production and which microorganisms might play a role in influencing secondary metabolite production.

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ARBUSCULAR MYCORRHIZAL FUNGI ENHANCES CORN TOLERANCE TO DROUGHT STRESS

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The expansion of drought-affected areas has a negative effect on crop yields and global food security. Water deficits are the most significant abiotic stress that limits the crop growth and development. In natural environments, mycorrhizal symbiosis allows plants to tolerate drought stress, but agricultural practices may reduce the efficacy of arbuscular mycorrhizal fungi. A greenhouse experiment is designed to determine how arbuscular mycorrhizal fungi associated with corn roots may enhance its water and nutrient uptake, when corn is grown with a water deficit or sufficient water, in soil with a history of fertilizer treatments. Soil will be collected from an established field experiment that received three types of treated wastewater biosolids (liquid mesophilic anaerobic digestate, lime-stabilized and composted), inorganic NPK fertilizer and no fertilizer, under monoculture corn production. Drought will be induced at three corn growth stages of corn (V₇– V₁₂, V₁₅– V_T and R₁– R₃). A conceptual model is presented describing how the corn tolerance to drought stress is influenced by interactions with arbuscular mycorrhizal fungi at the morphological, physiological and biochemical levels.

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SERBIAN LOWLAND TRUFFLE PRODUCING FORESTS DIFFER IN THEIR ROOT ASSOCIATED MYCOBIOMES

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Balkan Peninsula, an easternmost of Europe's three great southern peninsulas is a biodiversity rich area characterized by different terrain elevations, geological formations, as well as climatic zones. Forest ecosystems

in the Balkan peninsula are characterized as truffles producing habitats depending upon the dominant vegetation type. It is known that the aboveground vegetation as well as seasonal dynamics impacts the belowground microbial diversity. However, very little is known about root inhabiting/associated fungal communities of truffle producing habitats of Balkan peninsula in Serbian lowlands. Therefore, we investigated the impact of forest type and season on the root associated fungal diversity and community composition. The root samples were collected from soil cores of three different truffle producing forests at three time points representing different seasons. Genomic DNA was isolated from pooled root samples. We used Paired-end illumina sequencing approach to target the ITS2 rDNA fragment of the root associated fungal communities. We found a significant effect of seasonal variation on the fungal alpha diversity (ANOVA $p < 0.05$) in the white truffle producing forest. Beta diversity analysis indicated that the root associated fungal communities differ significantly between truffle producing forests (PERMANOVA $F = 4.8$, $df = 2$, $p = 0.001$). We conclude that the dominant tree species, season, and their interaction shapes the root associated fungal community composition in the lowland forest ecosystems.

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THE EFFECT OF FUNGICIDE ON ROOT AND LEAF ASSOCIATED FUNGI IN GLYCINE MAX.

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The environmental impacts of traditional agriculture are a growing concern. The overuse of pesticides and herbicides threaten fragile ecosystems such as wetlands and our freshwater resources. These synthetic chemicals can leach through soil and contaminate groundwater and linger in food products causing potential harm to wildlife and humans. The purpose of this project is to examine the fungal communities associated with *Glycine max* roots and leaves both pre and post-fungicide application using Illumina sequencing. A total of 3,627,618 sequences were generated from 72 samples of soybean roots and leaves. After quality filtering, 2,229,197 sequences were used to define Operational Taxonomic Units (OTU) resulting in 1990 OTUs. Forty-one percent of the fungal sequences belonged to the phylum Ascomycota this was followed by 6.4% in Basidiomycota. Soybean leaves both pre and post-fungicide application contained a higher number of sequences and OTUs as compared to roots. Preliminary results also suggest changes in dominant fungi in both roots and leaves after fungicide application. An unknown taxon of fungi in roots accounted for 43% of the total fungal sequences prior to fungicide treatment. After fungicide application this taxon decreased significantly to 8% and was replaced by *Fusarium* representing 32% of the sequences. In leaves *Didymella* (21.5%) was the most abundant fungal genus before fungicide use however after fungicide this fungal genus decreased to 1% and *Plectospharella* (10%) became the most abundant fungi. Fungal-plant interactions are a delicate and complex balance in a range of mutualistic to parasitic interactions. Understanding these interactions and the indirect impacts of fungicide on community composition will provide important insights into plant health.

P.108

PHYTOBIOME; TO BE FRIEND OR ENEMY IN TOMATO RHIZOSPHERE

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Microbiota associated with plants is crucial to modulate plant growth and health, however, it is not well characterized how microbial communities assemble in the vicinity of plants and play a role to promote specific plant function. In this study, we hypothesized that different soil microbiota contributes to forming tomato rhizosphere microbiome and may positively or negatively influence plant traits such as disease response. In order to test this hypothesis, we first established a system to study plant-microbiome interaction under defined soil condition by maintaining biological diversity to investigate microbiome effects on plant function. The potential effects of microbial fractions isolated from various natural or cultivated soils including paddy, alluvial, upland, and forest soil were tested on bacterial wilt (BW) caused by *Ralstonia solanacearum* occurrence in a susceptible and resistant cultivar of tomato plant under the established system. A specific upland microbiome conferred the BW-resistant cultivar Hawaii 7996 with the significant suppression of BW progress, whereas a specific forest one caused Hawaii 7996 more conducive to BW occurrence than non-microbiome treated control. Analysis of bacterial communities of tomato rhizosphere treated with different microbiomes displayed distinct community structure. For the selection of core microbiome contributing to disease response, we analyzed the rhizosphere microbiome of both cultivars inoculated by upland and forest microbiome at the different time points. In the analysis of beta-diversity, the bacterial community was clustered by the treatment of microbiome regardless of cultivars and time points. Interestingly, several OTUs were only observed in the rhizosphere treated with upland microbiome, showing less disease occurrence in the resistant cultivar of tomato. Taken together, the treated rhizosphere microbiomes isolated from natural field soil affect positively or negatively disease resistance of a resistant tomato cultivar.

P.109

BACTERIOPHAGE TO CONTROL TOMATO BACTERIAL WILT DISEASE AND ITS EFFECT ON TOMATO RHIZOSPHERE MICROBIOME

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Bacterial wilt disease of solanaceous crops caused by *Ralstonia solanacearum* is a devastating disease and difficult to control. As the environmental problem has become more prominent, bacteriophage that might be an environmentally friendly control method was selected to investigate their potential to manage bacterial wilt. Seventy-two phages were isolated from the field soils cultivating various crops using 5 different strains of *R. solanacearum*. We divided these 72 phages into ten distinct groups based on their host range and plaque size by the modified beta diversity method. Representative phages of each group were further characterized for their morphology and physiology via transmission electron microscopy, genomic characteristics, and phage stability at various temperature and pH. Three phages from each group were typical members of podoviridae with double strand DNA genome, and one phage was a typical member of inoviridae containing single strand

DNA genome. Phage application either single treatment or cocktail treatment into soils with tomato plants exhibited bacterial wilt prevention. In order to apply the bacteriophages as a biological control agent to the arable field, we tested the effect of bacteriophages on soil microbial community. Analysis of 16S rRNA gene amplicon sequencing revealed that application of a specific phage into tomato rhizosphere did not affect bacterial community structure compared to non-treatment control, although relative abundance of a few specific bacteria taxa has been altered. These results suggest that phages in this study may be suitable for controlling bacterial wilt in tomato plant.

P.110 (will also be presented as poster pitch)

MICROBIAL COMMUNITY NETWORKS ACROSS A SOIL DEPTH GRADIENT IN BIOFUEL CROPPING SYSTEMS

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Plant-associated microbes form complex and interconnected networks in the soil, which can be explored for sustainable crop development. Soils deeper than 20cm compose a poorly studied environment, but have important implications on switchgrass microbiome as *Panicum virgatum* develops deep root systems. Here, we utilize integrated resources from the Great Lakes Bioenergy Research Center to explore the microbial community composition over a soil-depth profile, with the ultimate goal of modeling how soil microbial networks affect sustainable biofuel production. The hypotheses we are testing are that: (1) microbial community diversity and network complexity will be lower in deeper soils; (2) higher connectivity between soil and root compartments across a depth gradient will be positively correlated to plant biomass and microbial biodiversity; (3) communities with multiple plant species will have more complex microbial community networks compared to monocultures; (4) occurrence and relevance of indicator taxa across soil depth will be correlated to higher network connectivity of the same genera. To test these hypotheses, soil cores with depths of 0 to 10cm, 10 to 25cm, 25 to 50cm, and 50 to 100cm were collected from plots with native switchgrass (*Panicum virgatum*, var. Cave-in-rock) monocultures, exotic hybrid popular ("NM-6", *Populus nigra* x *Populus maximowiczii*) monocultures, and restored prairie 17-species mix. Three representative samples were obtained from each of the 5 replicate plots, totaling 180 samples that were divided between soils and root fragments. Total DNA was extracted from these samples and amplified with the primer set 515F/806R targeting the V4 region of prokaryotic 16S rDNA and ITS1f/ITS4 targeting fungal internal transcribed spacer (ITS) rDNA region. A total of 45,720,600 raw reads were obtained 770 samples with the Illumina MiSeq platform. Preliminary analysis indicates a sharp decrease in microbial diversity and proportion of positive co-occurrences in the community networks as soil depth increases.

P.111

GENOTYPIC AND MANAGEMENT INDUCED MODIFICATIONS OF THE SPATIOTEMPORAL DEVELOPMENT OF RHIZOCTONIA SOLANI AG8 INFECTIONS

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Rhizoctonia solani is an economically important pathogen in the dryland wheat growing regions of the Pacific Northwest. Breeding programs seeking to develop tolerant varieties and farmers seeking to better manage for disease pressure could benefit greatly from the ability to quantify the development of lesions, spear tipping, and other symptoms. Three lines of wheat were grown in scanner based rhizoboxes and exposed to *Rhizoctonia solani* AG8. Images were taken at high spatial (< 1 mm) and temporal resolution (< 4hr) allowing for spatiotemporal analysis of root rot symptoms. The collected images showed the development of lesions and spear tipping on roots exposed to soil inoculated with *Rhizoctonia solani*. The percent lesion, number of spear tips, and total root area were quantified. Following the completion of the imaging the wheat roots were excavated and rhizosphere DNA was extracted. The methods and analysis presented here is potentially useful for increasing our basic understanding of root response to pathogens. In addition to the visual quantification of root rot symptoms, rhizosphere bacterial community and function for the tolerance and susceptible lines of wheat were assessed leading to an improved understanding of the role of bacteria in tolerant vs. susceptible wheat lines. The methods described here were successfully used to differentiate between wheat lines and seed treatments to better understand the role of management and genetics on root rot symptoms.

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MICROBIAL NETWORKS IN SOIL AGGREGATES AND THEIR DYNAMICS IN THE ROOT-SOIL CONTINUUM

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Plants roots provide soils with energy rich carbon (C) sources which trigger a cascade of abiotic and biotic reactions with consequences for soil structure. Soil microorganisms are the most important biological players in this process. We are studying microbial community dynamics induced by plant root exudates released into the soil with the objective of elucidating the interactions that shape microbial diversity and community function at the soil aggregate level. We extract microbial DNA from mg-sized soil samples and single aggregates thereby analyzing the microbial communities at the spatial scale in which microbial interactions are more likely to occur. In an agricultural setting, the root soil continuum is characterized by two contrasting gradients: a decline of energy rich C-sources, and an increase of nitrogen (N) with more distance from the root surface. The different C:N ratios impose selective pressures on the microbial communities in aggregates. Furthermore, it is anticipated that the mechanical effects of root growth and the input of energy-rich C and mucilage induces aggregate formation. We hypothesize that with increasing distance from the roots, the diversity of the microbial community as well as its carbon use efficiency in soil aggregates increases. Easily available C-sources in the vicinity of the root may favor competition, while more distantly from the roots more complex C-sources could foster cooperation. Microbial organization patterns will be assessed by network analyses. The abundance of

Bacteria, Archaea and *Fungi* is analyzed by qPCR and their diversity by sequencing 16S rRNA gene fragments and ITS regions. To appreciate the role of N in structuring communities in soil aggregates, we investigate the abundance and diversity of genes connected to the soil N cycle. Model systems are utilized including soil columns and rhizotrons, the latter to assess spatial heterogeneity by physical, chemical, and biochemical methods, and visualization techniques.

P.113 (will also be presented as poster pitch)

THE UNIQUE ILLUSTRATION OF HOLISTIC RHIZOSPHERE BOUNDARIES BY COUPLING ZYMOGRAPHY AND ¹⁴C IMAGING

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The rhizosphere is an important hotspot concerning nutrient mobilization with high intensity of biochemical processes. Root morphology plays a critical role with respect to exudation and nutrient acquisition. Enzymes originated from both roots and microorganisms are the main drivers of SOM decomposition. Thus, understanding the photoassimilate allocation into roots and their localization is crucial for characterizing the spatial distribution of enzyme activities and nutrient mobilization.

Three maize genotypes – wild type, root-hair less mutant (*rth3*) and mutant with modified root exudate metabolite composition (*bx1*) were chosen to investigate the effects of root morphology and exudate composition on the spatial distribution of β -glucosidase activity and estimate the extent of rhizosphere for root exudates. To visualize these spatial patterns, we combined two imaging methods: 1) Zymography for spatial distribution of β -glucosidase activity and 2) ¹⁴C imaging to assess the root derived ¹⁴C release (i.e. root exudates).

The extent of the rhizosphere for β -glucosidase in wild type maize across the root was 20-25% broader and its average activity was 15-20% higher than in the rhizosphere of both mutants. This confirms the distinguished role of root hairs on enzymatic mobilization of nutrients, and point out on the importance of enzyme expression as a plants strategy for nutrients' mining.

For the first time, we visualized by ¹⁴C imaging that genotypes performed specific exudation strategy: 1) preferably via root tips (wild), 2) from the whole root system (*bx1*) or 2) combining both strategies (*rh3*). Thus, the rhizosphere boundaries depend on genotypes, which define root morphology and exudation.

In conclusion, we defined the critical role of root hairs in localization of exudates, as well as enzymes spatial distribution and their activity per root area. These data allowed finding the holistic borders of the rhizosphere taking into account the biochemical processes.

P.114 (will also be presented as poster pitch)

DO LEGUME-BASED INTERCROPS IMPROVE SOIL FAUNA AND SOIL MICROBIAL DIVERSITY? EXAMPLE OF THE COWPEA-CASSAVA INTERCROPPING SYSTEM IN NORTHERN VIETNAM (YEN BAI PROVINCE)

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Industrial agriculture has allowed food safety through the crop homogenization and the massive use of pesticides and chemical fertilizers. This intensive agriculture has positively influenced the crop productivity but has also negatively affected the environment with direct consequences on soil health and productivity. The importance of the micro and macro organisms in the sustainability of soil fertility is widely recognized. Soil fauna represents 23% of the global fauna and contributes to most of the ecosystem services such as porosity, soil aggregation, control of the microbial activity and plant protection against pests and diseases. To mitigate the collateral effects of intensive agriculture on soil productivity in South East Asia, agro-ecological practices are currently promoted, including the use of legumes in intercropping systems. We studied the effect of the intercropping system cassava-cowpea (*Vigna unguiculata* L.) on the soil biotic compartment in Yen Bai province, a mountainous region in the North of Vietnam. Intercropping resulted in the increase of macro fauna richness and evenness. Intercropping system also significantly enhanced both diversity and abundance of soil micro fauna. The high throughput sequencing analysis of the microbial community showed that bacterial communities' abundance and richness were significantly higher in intercropping system than in mono-cropping fields. However, intercropping did not significantly affect the fungal communities and the soil parameters including pH and organic matter. Our results highlight the importance of the promotion of agro ecological practices such as legume intercropping systems in the Highlands in Northern Vietnam where the sustainability of the ecosystem is very fragile.

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SPATIAL DISTRIBUTION OF ROOT EXUDATES AND ITS EFFECTS ON MICROBIAL FUNCTIONS UNDER DROUGHT

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Root hairs and rhizosphere water content play an important role in exudation and shaping the profiles of microbial activity and functionality in the rhizosphere. Thus, understanding the photoassimilate allocation into roots and their localization is crucial for characterizing the spatial distribution of enzyme activities and nutrient mobilization in response to water stress.

For the first time we combined three imaging techniques, ¹⁴C imaging, zymography and neutron radiography to illuminate how the rhizosphere self-regulation (for example changes in exudation or root hairs proliferation) responds to drought and affects spatial distribution of enzyme activities in the rhizosphere. To this end, two maize varieties – wild type (with root hairs) and mutant type (without root hairs) – were grown under optimum and drought conditions for 3 weeks. Both varieties allocated similar amounts of ¹⁴C into roots irrespective of the water content. Root elongation decreased by 50% in dry soil leading to reduced longitudinal rhizosphere extension. Root hairs maintained broader diffusion of exudates and higher enzyme activity even under water limitation. Similarly, the extent of the rhizosphere for β-glucosidase in wild type maize across the root was 20 and 30% broader than the mutant in wet and dry samples. Rhizosphere water content was not affected by drought, presumably because of the high-water retention by mucilage. However, soil water content under wild type was 20% higher than under hairless mutant.

We conclude that root hairs enhanced the diffusion of exudates into the soil by releasing mucilage which increases the local water content in the rhizosphere. Hereby, they stimulate interactions with microorganisms and contribute to a higher nutrient accessibility. Thus, the decrease in rhizosphere water content reduced diffusion of exudates and enzyme activity. Our multiple-imaging approach allowed illuminating the holistic boundaries of the rhizosphere properties and processes.

P.116

UNDERSTANDING THE FUNDAMENTAL PROPERTIES OF ROOT EXUDATES UNDER DRYING

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Plant roots exude a substantial proportion of their fixed carbon as root mucilage, a viscous polysaccharide gel, which plays a number of important roles in the rhizosphere. Root mucilage is hypothesised to act as a lubricant for the growing root, to influence soil structure and stability as well as hydraulic conductivity and it may assist in drought tolerance by holding water close to the root surface. Mucilage also interacts with microbial communities in the rhizosphere and helps to mobilise strongly bound soil nutrients.

However, the properties of root mucilage change entirely on drying: rather than holding water close to the root surface, dried mucilage can create hydrophobic regions in soil, slowing later rewetting of the rhizosphere. Mucilage drying can also affect soil stability and aggregation as the viscous gel transitions into a solid, robust film. Thus, understanding when and how this transition occurs from first principles is critical for a more profound understanding of the generation of the rhizosphere.

Therefore, our objective is to characterise the properties of root mucilage, both hydrated and drying. We use chia seed exudate as an analogue for root mucilage as it has a similar chemical composition and physical properties. We use experimental methods including light microscopy to obtain key measurements such as

contact angles and study the fundamental properties of the material. We quantify critical time points and water contents associated with the gels transition from viscous liquid to rigid solid, which offer insights into the dynamic functions of the rhizosphere at different times. We combine experimental data with simulations of the drying mucilage based on first principles in order to understand the drying behaviour of root mucilage at a fundamental level.

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SIMULATING ROOT WATER UPTAKE REGULATION UNDER DROUGHT STRESS USING DUMUX -CROOTBOX

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Plant transpiration and root water uptake are dependent on multiple traits that interact with site soil characteristics and environmental factors such as radiation, atmospheric temperature, relative humidity, and soil-moisture content. Models of root architecture and functions are increasingly employed to simulate root-soil interactions. Root water uptake is thereby affected by the root hydraulic architecture, the soil moisture conditions, soil hydraulic properties and the transpiration demand as controlled by atmospheric conditions. Stomatal conductance plays a vital role in regulating transpiration in plants. Isohydric plants follow the strategy to close their stomata in order to maintain the leaf water potential at a constant level, while anisohydric plants leave their stomata open when leaf water potentials fall due to drought stress. Modeling the stomatal regulation effectively will result in a more reliable model that will regulate the excessive loss of water. We performed the simulations of plant water uptake and transpiration on a DUNE-based simulator DuMu^x. It simulates the flow and transport processes in porous media that can represent the porous medium (including soil) with embedded hierarchical biological networks (in this case, root system). We implemented hydraulic and chemical stomatal control of root water uptake in DuMu^x following the current approach where stomatal control is regulated by simulated water potential and/or chemical signal concentration. We then performed a mesh convergence study in order to verify the accuracy of our results. Finally, we conducted the simulations for different scenarios to study the effect of hydraulic and chemical regulation on root system performance under drought stress.

Future work will include incorporating further modeling capabilities into DuMu^x which will facilitate the consideration of further soil and rhizosphere processes in the simulations, such as nutrients, root exudes or soil microorganisms.

Keywords: Transpiration, root-soil interaction, root water uptake, stomatal conductance, hydraulic control, root-soil modeling

P.118 (will also be presented as poster pitch)

A MOLECULAR INSIGHT INTO THE RHIZOSPHERE WITH UNRIVALLED CHEMICAL, TEMPORAL AND SPATIAL RESOLUTION

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Plant-microbe interplay in the rhizosphere generates multi-faceted chemical gradients. The soil solution is a crucial component of the rhizosphere, where chemical gradients of organic molecules first develop upon growth of roots, introduction of plant-derived carbon and microbial turnover. Studying these gradients requires high resolution both in time and space as well as high chemical specificity to resolve the multitude of compounds. While methods already exist for probing rhizosphere soil solution for inorganic components, methods to study gradients of organic molecules are mostly limited to bulk properties or target analytes.

Here we present a novel method that combines time-resolved collection of soil solution samples via micro-suction cups in the rhizosphere with ultrahigh chemical resolution provided by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to unravel developing pattern of soil solution organic matter.

Zea mays plants were grown in soil columns for three weeks and soil solution samples of undisturbed root-soil system were collected once a week. Growth of the root system and sampling locations were followed by X-ray computed tomography (X-ray CT). Soil solution samples were subjected to a sample preparation workflow and subsequently analysed by FT-ICR-MS.

The sample preparation was optimised to yield concentrates with low salt concentrations, allowing analysis of minute amounts of organic carbon within the soil solution samples. Given the high background concentration of organic carbon, FT-ICR-MS enabled to distinguish root derived molecules from soil organic matter based on their exact masses. Molecular formulas for the root derived molecules could be calculated showing distinct chemical characteristics. X-ray CT analyses enabled relating the results from the chemical analysis to distance from the root and root age.

Our method is thus capable to show the changing pattern of soil solution organic matter during the early rhizosphere development, closing the knowledge gap between root exudates, soil chemistry and microbial processes.

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OAK FINE ROOTS AND ECTOMYCORRHIZAL COMMUNITIES UNDER CHALLENGE OF ABIOTIC ENVIRONMENT

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Encroachment of abandoned agricultural land with woody perennials is an important carbon sink. In Slovene Karst area, human activities in the past have resulted in huge soil loss. Area has been colonized predominantly

by pubescent oak (*Q. pubescens* Willd.) with accompanying trees and shrubs, covering approximately 60% of land. Abiotic factors are strongly limiting vegetation thriving in this area, with calcareous bedrock being an important contributor to drought. With increasing extreme weather events, most importantly drought, vegetation survival in this area is under threat, as well as associated ecosystem services. To evaluate the response of trees to environmental conditions the knowledge of structural and physiological changes is crucial and must include tree mycorrhizal symbionts. To investigate morphological response of pubescent oak fine roots and associated ectomycorrhizal fungal (EMF) communities to environmental conditions in the area, soil cores were sampled from June 2016 till May 2018 on three plots (calcareous – rendzic leptosol, calcareous - rendzic leptosol + rainfall exclusion, eutric cambisol). Plot with rainfall exclusion simulated climate change with longer drought periods. From each soil core, ectomycorrhizal morphotypes associated with oak roots were isolated, morpho-anatomically characterized, and sequenced using ITS1/ITS4 markers. Assembled sequences were identified using UNITE database and molecular identification was compared and combined with morpho-anatomical identification. Tree roots were scanned, and scans analysed with WinRhizo to obtain morphological parameters. Our preliminary results showed the overall effect of season on the number of root tips, while root biomass, root diameter and specific root length were affected by plot and season. No effect was observed on root length. The lowest general EMF richness and the lowest seasonal variability in species richness was observed on calcareous plot with rain exclusion. Percentage of vital ectomycorrhizal tips was dependent only on season, with peaks in late spring/early summer.

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ASSESSING THE EFFECT OF SOIL TEXTURE ON DYNAMIC MAIZE ROOT GROWTH BY MAGNETIC RESONANCE IMAGING

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Soil texture is an important determinant of crop yield. Soil texture is determined by particle types and sizes, which influence the physical, chemical and biological environments experienced by roots. Dynamic root growth is a crucial mechanism by which roots respond to soil texture. It enables roots to penetrate different porosities, access water and nutrients and facilitate interactions with beneficial microorganisms. Although root traits are increasingly studied, we have an incomplete knowledge of spatiotemporally resolved growth patterns in response to soil type. Most studies rely on destructive sampling methods, which do not allow individual root growth to be tracked in space and time to research root dynamics. Therefore, we employed non-invasive Magnetic Resonance Imaging (MRI) to investigate dynamic root traits of maize (*Zea mays*) in response to different soil textures. MRI allows for 3D monitoring of root growth in its natural environment over time. Based on previous research, we expected restricted root exploration in compact loamy soils, and faster and finer root growth in porous sandy soils. Maize plants were grown in loamy soil, a sandy-loam (containing 16.7% loam), and a silty-sand soil. Roots were scanned using MRI at 2, 6, 9, 13, 15 and 20 days after sowing (DAS); then

harvested at 21 DAS. Root growth was visualized successfully by MRI in the three soil types. Dynamic root traits quantified using the MRI analysis software NMRRooting will be presented, along with WinRHIZO data obtained after harvest, to validate the MRI. 3D imaging by MRI is a promising technology to define critical time points in root development and thus rhizosphere organization. Spatiotemporal maps of root growth enable targeted sampling of root function and structure, plus associated microorganisms, to understand dynamic processes in roots and rhizospheres and their links to shoots.

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SPATIAL PATTERNS IN THE ROOT-ASSOCIATED MICROBIOTA OF APPLE TREES VARY IN DEPENDENCE ON ROOT SECTION

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The rhizosphere harbors highly specific microbial communities, which have the potential to influence plant growth and development. Plants specifically select certain species from the surrounding soil, which thrive in the root-associated soil. Some of these microorganisms even enter the root and establish an endophytic lifestyle. The root system of trees is quite complex and it is assumed that rhizodeposition processes mainly occur at the root tips and in the elongation zone. Older root sections become suberized and thus are considered less relevant concerning carbon release into the rhizosphere and nutrient uptake. It is therefore hypothesized to find different microbial communities along different root regions, which were classified in this study using root diameter as proxy. In order to evaluate these community differences, four commercial apple trees (*Malus domestica* Borkh.) were excavated and the root system was sampled systematically with high spatial resolution and in dependence on root diameter. Effects on the root-associated microbiota were evaluated in two compartments: the microorganisms residing inside the roots and closely attached to the root surface were defined as tightly associated, while the microorganisms loosely bound to the rhizoplane and microorganisms found in the rhizosphere were defined as the loosely associated microorganisms. All samples were subjected to bacterial community analysis based on 16S rRNA gene amplicon sequencing. Root diameter did not significantly affect variation in bacterial alpha-diversity. However, the individual trees slightly influenced the diversity of the loosely though not the tightly associated microorganisms. The key factor for differences in beta-diversity of the tightly associated fraction was indeed the root size, as hypothesized. However, differences in the community of loosely associated bacteria was greatest in dependence of the individual tree. Thus, specific root sections have a stronger impact on endophytic and tightly attached rhizoplane bacterial community composition than on the loosely associated rhizosphere bacteria.

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CHARACTERIZING THE INFLUENCE OF SOIL RECRUITMENT POOLS ON RHIZOSPHERE COMPARTMENTALIZATION IN MAIZE SEEDLINGS

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Achieving sustainable management in agroecosystems to foster resiliency and promote crop yields is a major challenge of the 21st Century. Crop diversification is described as one management strategy that may support more resilient soil environments capable of withstanding abiotic and biotic stress. Alterations in above-ground inputs contribute to changes in below-ground habitats and shifts in microbial community structure. Our research is interested in linking changes in microbial community composition and soil habitat to broader ecological processes in order to ascertain the impact of management strategies on agroecosystem productivity and sustainability. To this end, we investigated the role of crop rotation and nitrogen fertilization history on soil microbial recruitment pools. We define recruitment pools as the microbial community associated with a soil that roots may access upon germination and recruit for rhizosphere and root colonization. Using soil from a long-term field site in Eastern Nebraska, we set up a greenhouse study to explore the ecological process of assembly of the maize rhizosphere during early growth stages. We evaluated four crop rotations, each with three nitrogen rates, and employed a novel sampling approach that defines 'recruitment pools' and delineates the rhizocomplex into two fractions: the loosely adhered root-influenced soil and the tightly adhered root-associated soil. Here, we will present the first part of a larger study, comparing soil recruitment pools to that of soil in the loosely adhered root-influenced fraction. Preliminary data includes fatty acid profiling (FAMEs), plant growth metrics, and characterization of nutrient pools in the soil environment. While not part of our results at this time, future analysis will incorporate amplicon sequencing, metagenomic profiling, and enzyme assays to address the diversity and potential functional roles of microbial communities across a range of crop and nitrogen histories.

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A NEW NEUTRON TOMOGRAPHY APPROACH RELATING SPATIOTEMPORAL HYDROCHEMICAL RHIZOSPHERE PATTERNS AND 3D ROOT SYSTEM ARCHITECTURE

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Dynamic processes occurring at the soil-root interface crucially influence soil physical, chemical, and biological properties at local scale around the roots that are technically challenging to capture *in situ*. Combining 2D optical fluorescence imaging, neutron radiography and 3D neutron computed laminography, we have tested an enhanced imaging approach capable of simultaneously quantifying H₂O-, O₂-, and pH-distribution around living plant roots in soil. By additionally capturing the root system architecture in 3D the observed 2D patterns

of root distribution, respiration, exudation, and water uptake are provided at high temporal and spatial resolution and now could also be used for explicit numerical modelling.

Planar optodes were attached to opposite inside glass walls of soil-filled rhizoboxes (15 x 15 x 1.5 cm³) to capture pH and O₂ maps of a root system by fluorescence imaging while the 2D water distribution was measured by neutron radiography. Complementary, neutron computed laminography, a tomographic approach specially adapted to samples with large lateral extension, was applied to visualize the 3D root system architecture. Neutron computed laminography made it possible to quantify the distance of roots from the container walls and thus from the optodes, which enhances the interpretation of observed pH and oxygen patterns. Spatiotemporal changes in water content distribution, oxygen concentration and pH values can be interpreted also with respect to root age. The measured 3D root structure could be directly implemented in a reactive transport model that can be calibrated via the fluorescence imaging results. The model then can yield a 3D representation of dynamic rhizosphere patterns and biogeochemical gradients and thus complement the measurements.

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FUNNELIFORMIS MOSSEAE ALTERS SOIL FUNGAL COMMUNITY DYNAMICS AND COMPOSITION DURING LITTER DECOMPOSITION

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Although arbuscular mycorrhizal fungi (AMF) are believed to be non-saprophytic, recent studies have indicated that AMF are able to influence litter decomposition through interacting with the soil fungal community. However, it remains unclear exactly which constituent groups of the soil fungal community respond to AMF during litter decomposition, and in what ways. In order to fill this knowledge gap, we investigated the effect of AMF on soil fungal communities in a subtropical forest in southwestern China. Our experimental set-up included a dual microcosm unit with two treatments: inoculated with AMF (AM) and uninoculated (NM). Destructive soil sampling was carried out at different times (T₀, T₉₀, T₁₂₀, T₁₅₀ and T₁₈₀) and Illumina sequencing was used to detect changes in soil fungal community composition. We found that the composition and operational taxonomic unit richness of the fungal community, at higher taxonomical levels (e.g. phyla, order), remained stable across treatments. However, the relative abundance of some key genera including *Mycena*, *Glomerella*, *Pholiotina*, and *Sistotrema* were significantly affected by AMF inoculation. Soil fungal community structure was also significantly altered by AMF inoculation during the later stages of litter decomposition, but the diversity of the soil fungal community was unaffected. Our study provides new insight into understanding the interaction between AMF and soil fungal communities during litter decomposition.

P.125

INDOLE-3-ACETIC ACID CATABOLISM IN *B. JAPONICUM* E109 AND ITS IMPACT ON SOYBEAN INOCULATION

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Bradyrhizobium japonicum E109 has been one of the most used rhizobacteria for soybean inoculation in South America during the last 40 years. The genome analyses of E109 showed that besides nitrogen fixation, these bacteria contain several genes related with phytohormone biosynthesis, particularly indole-3-acetic acid (IAA). However, it is not possible to quantify this compound under any experimental conditions, mainly due to the bacterial capacity to degrade it. The degradation kinetics of E109 showed a higher activity during exponential growth phase with a saturation behavior above to 0.5 mM IAA in YEM liquid culture medium. A second genome analysis revealed the presence of a catabolism cluster similar to that previously identified in *Pseudomonas putida* 1290, but although the *iaca* gene product is responsible for IAA degradation in *P putida*, it is not in *B. japonicum* E109. Conversely, the homologous *iacc* of E109 encoded for a 3-phenylpropionate dioxygenase subunit α , the main responsible for IAA degradation in E109 forming a particular cluster together with *iaccF* and two genes with unknown function. The *iacc* mutation rendered E109 unable to degrade IAA ~~and~~ under *in vitro* and *in vivo* conditions, confirming the enzyme role. Besides inducing bacterial catabolism, the addition of 40 $\mu\text{g}\cdot\text{ml}^{-1}$ IAA in YEM liquid medium increased both E109 and mutant $\Delta iacc$ viability on soybean seeds 4 hours and 6 days after inoculation. Additionally, under *in planta* conditions, soybean nodulation and the number of nodules in primary roots increased significantly when adding the hormone to $\Delta iacc$ —suggesting a regulatory role of *iacc* in the symbiosis establishment.

P.126 (will also be presented as poster pitch)

LEADING BIOLOGICAL SOIL HEALTH: AN INDUSTRY PERSPECTIVE

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The depletion and degradation of arable soils around the world constitutes a significant threat to sustainable food supply that is required to feed a rapidly increasing global population. While intensive agricultural practices have provided increased crop yields, they have also led to a significant increase in soil erosion, reduced water efficiency, and a disruption to the soil microbiome essential for optimal soil health and plant production. In response to this situation and with pressure from food chain stakeholders, both public and private sector entities are collaborating to conduct scientific research & development initiatives to employ soil microorganisms that naturally replenish and maintain healthy soils, such as arbuscular mycorrhizal fungi. These fungi form hyphae that produce a glycoprotein called glomalin that binds soil particles into stable aggregates, which mitigate soil erosion, improve water infiltration, and restore soil water holding capacity. The short- and

long-term benefits of arbuscular mycorrhizal fungi and their contribution to soil health will be reviewed. Valent BioSciences is leading the commercialization of arbuscular mycorrhizal fungi with novel, stable product formulations that afford application consistency, and can be easily integrated into current agricultural production practices.

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DO COMMERCIAL ARBUSCULAR MYCORRHIZAL FUNGAL INOCULANTS IMPACT INDIGENOUS AMF COMMUNITIES IN AGRICULTURAL SOILS?

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Use of commercial non-indigenous arbuscular mycorrhizal fungi (AMF) as bio-fertilizer inoculants for crops is increasing worldwide. There is, however, a lack of information on survival of introduced inoculants and consequences on indigenous AMF communities. To address this knowledge gap, a three-year field study was conducted at four locations across the semi-arid to sub-humid climate zones of Saskatchewan, Canada. At each of site, a commercial AMF inoculant (*Rhizophagus irregularis*) was applied to open-ended soil cores in which host plants (pea-wheat-pea rotation) were subsequently grown during three seasons. Pyrosequencing allowed phylogenetic analyses of AMF operational taxonomic units (OTUs) specific to the introduced inoculant and indigenous AMF taxa. The inoculant was still detected at two field sites (Swift Current-Brown soil and Outlook-Dark Brown soil) after three growing seasons, but persistence at two other sites (Scott-Dark Brown soil and Melfort-Black soil) was limited. Inoculation resulted in significant suppression, displacement, and alteration of some minor indigenous AMF taxa (*Rhizophagus*, *Septoglomus*, *Diversispora*, and *Archaeospora*) in the first cropping season. The persistence of the inoculant and impact on the abundance and diversity of indigenous AMF taxa were significant and related to specific field sites. Inoculant persistence and impacts on indigenous AMF communities were influenced by soil and environment interactions.

Keywords: arbuscular mycorrhizal fungus, inoculant, persistence, and pyrosequencing

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STUDY OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN THE RHIZOSPHERIC SOIL OF LITCHI AND MANGO ORCHARDS

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Understanding biotic and abiotic factors that impact arbuscular mycorrhizal (AM) fungal communities is of great importance to better utilize AM resources, thereby maintaining or improving agroecosystem sustainability. Mango and litchi are important woody mycorrhizal fruit trees in southern China, but there is little information on AM fungi associated with these two fruit crops. Using Illumina high-throughput sequencing we analyzed AM colonization, spore density, and community composition and diversity in the rhizospheric soil from 16 orchards of mango and 16 orchards of litchi in southern China. The results were

correlated to geographical position, soil chemistry, and manure input. In general, the majority of AM fungi OUTs belonged to the families *Paraglomeraceae* and *Glomeraceae*, but *Archaeosporaceae* was more abundant in rhizospheric soil of litchi orchards. The sampling sites were in areas of medium or low altitude, and there was a significant positive correlation between altitude and AM fungal richness. We found that higher soil nutrient content, particularly available-P and $\text{NH}_4^+\text{-N}$, was negatively correlated with arbuscular mycorrhizal colonization. In addition, our results showed that some AM genera respond differently to environmental variables. Finally, structural equation modelling (SEM) demonstrated that increasing manure input could decrease arbuscular mycorrhizal colonization but had no effect on richness of AM fungi. Our study elucidated some important factors that influence AM fungal communities and diversity in mango and litchi orchards, and provides a framework for adjusting fertilization regimes in consideration of AM symbiosis.

Keywords: AMF; mango; litchi; manure

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STREPTOMYCES AS A PLANT'S BEST FRIEND?

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Plant roots release diverse compounds to create a unique environment, the rhizosphere, in which a vast amount of microorganisms find their niche for growth. A subset of these microorganisms, commonly referred to as PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR), greatly contributes to plant health and productivity in various manners. Previous studies revealed the potential of *Streptomyces* sp. as potent plant growth-promoting bacteria in different plant hosts. In my project, we work with a *Streptomyces* strain which was identified because of the strong growth promoting effects on juvenile growth of the crops maize and wheat, suggesting potential agronomical relevance. However, to fully exploit its potential in agriculture, it is important to understand the plant developmental networks that are activated by these bacteria to increase plant growth and productivity and how these networks relate to the various signals produced by these bacteria. To obtain detailed insights into the plant growth-promoting activities of this *Streptomyces* strain, we focus on the interaction with the model plant *Arabidopsis thaliana*. Inoculation of *Arabidopsis* with the *Streptomyces* strain results in a strong growth promoting effect on the root as well as the shoot. The root architecture does not change significantly after inoculation, but the increased fresh weight can be explained by an increase in root diameter. In addition, after inoculation, there is a significant increase in both the number of leaves and the leaf size. What the underlying basis is of this PGPR effect is so far not known. In order to get insights into this matter, we take a combined physiological, molecular and functional approach to understand the bacterial colonization strategies and the plant molecular networks that determine the PGPR effect.

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IDENTIFYING MICROBIAL GENES ALONG WITH COMMUNITY PROCESSES THAT INFLUENCE COMPETENCE OF PLANT-BENEFICIAL MICROBES

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In nature, plant roots live in close association with countless microbes, also known as the root microbiome. Beneficial microbes provide plants with important services, such as enhanced nutrient uptake and protection against diseases. Increasingly microbial products, bioinoculants, are registered for biofertilization and biocontrol purposes. Despite their promise under controlled conditions, bioinoculant efficacy can vary in the field. This research aims to identify genes important for rhizosphere competence and uncover the effect of bioinoculant introduction on the resident microbiome and on plant fitness, and vice versa. We will introduce a random-barcoded transposon mutant library of plant growth-promoting rhizobacteria to *Arabidopsis thaliana* seedlings grown in soil. By measuring mutant frequency via next-generation sequencing we aim to identify genes important for root colonization in the context of the resident microbiome. Bulk soil will be sampled similarly to identify plant-dependent effects specifically. We will perform shotgun metagenomic sequencing to identify and functionally characterize the microbial community followed by metatranscriptomics to investigate microbial activity. With community network analysis on the level of species as well as genes we aim to investigate which niche introduced rhizobacteria take in resident microbial communities. By analysis of a proxy for the amount of clustering of closely related species, we can then disentangle the relative contribution of different community assembly processes. To establish our experimental system, we first studied the effects of root extraction methods on community composition analyses in the rhizosphere. Roots of *Arabidopsis* plants were sampled using four different extraction methods differing in the amount of attached soil. All treatments showed a clear rhizosphere effect, the strongest of which was found for two distinct washings. In sum, this work will further our understanding of the molecular and ecological processes underlying microbial community assembly and highlight important features of rhizosphere competence; prerequisites for successful bioinoculant application in the field.

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ROOT ENDOPHYTIC SERENDIPITA SPECIES AFFECT GROWTH AND NUTRIENT CONTENTS IN TOMATO PLANTS

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Root endophytic fungi have recently gained more and more attention due to the ability of some representatives to impact positively on crop plants. Among the recently studied groups of root endophytic fungi is the family of Serendipitaceae. *Serendipita indica*, isolated from a *Funneliformis mosseae* spore, originated from a desert soil in India is the most well studied member of this family and exerts positive effects on plant growth and health in many different plant species. Two other members of this family including *Serendipita williamsii* (Sw) and *Serendipita herbamans* (Sh) were isolated from European sites and might be more suitable for the application

in Europe; however, the potential of these two European isolates to influence plants is yet to be explored. Thus, this study aims to test the effects of *Sw* and *Sh* on the growth and nutrient contents of tomato as among the most consumed vegetable crops in Europe. In spring 2018, a greenhouse experiment was conducted using tomato plants with the following factors: (1) *Serendipita* spp. including *Sw* and *Sh* and (2) nutrient concentrations including low and high phosphorus (P) and low and high nitrogen (N). The results demonstrated that only *Sh* inoculated plants showed higher dry weights of tomato shoots when P fertilization was high as well as higher root length when N fertilization was low. With regards to the nutrients accumulated in the shoots, macro- and micronutrient concentrations remained unchanged even with the inoculation of *Serendipita* spp. under low and high P fertilization. However, when the factor N fertilization was varied, *Sw* inoculated plants showed an increase of potassium and zinc contents especially under low N fertilization. Hence, *Sh* is regarded as suitable growth promoting and *Sw* as nutrient accumulating root endophytes of tomato but their impacts are reliant on the concentration of nutrients fertilized to plants.

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PRODUCTION OF ANTIFUNGAL COMPOUNDS PRODUCED BY BACILLUS STRAIN ON MODIFIED BIOREACTOR TO CONTROL RHIZOCTONIA SOLANI

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A collection of twenty Gram-positive bacilli isolated from rhizosphere of common bean plants, showed plant growth promotion including high antagonistic activity against soil-borne plant pathogens like *Sclerotinia sclerotiorum*, *Fusarium oxysporum* and *Rhizoctonia solani*. The antagonistic activity was related to production of several substances such as volatile antifungal compounds, siderophores, antibiotics and lytic enzymes production. Culture filtrate previously obtained by acidification of cell free supernatant of *Bacillus* IcBac2.1 strain showed strong mycelial growth inhibition against the phytopathogens tested. Antifungal antibiotic compounds belong to peptide group. Lytic enzymes belong to cellulose, protease and amylases. In order to optimize the production of antifungal compounds by IcBac2.1 strain was investigated the use of a modified bench-top bioreactor with different aeration and agitation rates in order to control *R. solani*. Antifungal compounds were produced at 72 hours in mineral broth supplemented with starch (20 g l⁻¹) and yeast extract (8 g l⁻¹). At the bioreactor level, the results indicate that the modified bioreactor with a foam collector allow to produce high concentration of antifungal compounds compared to a simple reactor. Parameters at 400 rpm and 1 vvm allow to produce crude antifungal compounds concentration (7.224 g l⁻¹), bacterial growth (1.932 g l⁻¹) and spore production (4.2E+08 UFC/ml) compared to others parameters tested. Phylogenetic analysis confirmed that strains were related to *Bacillus* and *Paenibacillus* genus. The production of different antifungal compounds by *Bacillus* IcBac2.1 strain using mineral broth on modified bioreactor represent a low cost technology to crop protection.

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THE IMPACT OF NITROGEN-MODIFIED LIGNITE GRANULES ON ROOT GROWTH AND MYCORRHIZAL COLONIZATION OF TRITICUM AESTIVUM

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Inappropriate soil management practices and soil degradation, more recently reinforced by climate change, account for a worldwide loss of soil organic matter (SOM). SOM is even completely missing in soils derived from mine site reclamation. Envisaging an increasing demand for fertile soils, technologies for the restoration of stable soil organic matter and, hence, biological, physical and chemical soil functions are needed. To reach this aim organic soil amendments such as biochar and Terrapreta have more recently come into focus. However, properties of such products may highly vary and their availability may be restricted. In contrast, granulates produced from chemically modified lignite allow for the provision of a homogenous product in large quantities. In a greenhouse experiment, the effects of a lignite-derived nitrogen-enriched soil amendment (NOVIHUM®) on root growth and mycorrhizal colonization of spring wheat (*Triticum aestivum* L.) were tested in a sandy, nutrient and organic matter-limited substrate from a post-mining site in Eastern Germany. We hypothesized that the organic granules increase plant growth and improve the colonization of roots with arbuscular mycorrhizal fungi (AMF). The experimental design comprised the following treatments: (i) sandy soil without inoculation (control), (ii) without inoculation but NOVIHUM application, (iii) NOVIHUM application plus AMF inoculation, (iv) treatments with only AMF inoculation, (v) mineral nitrogen application, (vi) mineral nitrogen plus AMF. Roots were analyzed for biomass, length, diameter, mycorrhizal colonization and shoot biomass and shoot total nitrogen concentration was determined.

NOVIHUM application significantly increased the mycorrhizal colonization of roots and enhanced shoot growth and nitrogen uptake in contrast to the other treatments. Root length was significantly reduced in all treatments compared to the control. Results suggest a positive impact of NOVIHUM on mycorrhizal activity accounting for the enhanced plant growth under nutrient-limited conditions. Further studies are needed to verify whether these findings will be persistent.

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MIND YOUR ROOT-MATES: TRAITS TO INFILTRATE IN THE RHIZOSPHERE ECOSYSTEM

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With an ever-increasing human population worldwide, modern agriculture must face the challenge of boosting crop yields while decreasing synthetic inputs. One of the main solutions is the use of beneficial microbes as biofertilizers or biocontrol agents, collectively referred to as bioinoculants.

Plants however, distort soil microbial communities, yielding a highly complex and competitive ecosystem where bioinoculant survival is compromised due to the many barriers imposed by this environment - e.g. a locally

adapted microbiome, predation, and the presence of antimicrobial compounds -. Consequently, the effects are unpredictable and are frequently absent or rapidly disappear after addition.

To design effective bioinoculants it is thus crucial to understand the genomic traits that grant microbes the ability to infiltrate, colonize, and persist in a natural rhizosphere ecosystem.

Here, we construct libraries of transposon insertional mutants in bioinoculants to map the genes that contribute to their survival in natural rhizosphere communities. These libraries are built by random insertion of a barcoded transposon, and our focus relies currently on building the transposon delivery vector. A random, 30-bp fragment in the barcode is used to uniquely identify mutants, so our main concern is to keep the diversity of barcodes as high as possible along the process of vector construction. This will ensure sufficient sequence distance among barcodes to differentiate every mutant in the population.

Once constructed, the library is applied to the roots of *Arabidopsis thaliana* plants growing in natural soil. The mutants' profile is then traced by reading the barcodes through NGS and compared to the profile in bulk soil. The knowledge on the differentially selected genes in the rhizosphere ecosystem will enable us to unearth the fundamental requirements for survival in a natural root environment and subsequently develop more effective bioinoculants to ensure future crop production, sustainability, and safety.

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CURRENT SEASON NITROGEN FERTILIZATION RATE, NOT PRIOR N HISTORY, DICTATES EXTRAMATRICAL HYPHAL BIOMASS OF ARBUSCULAR MYCORRHIZAL FUNGI IN SOIL AT MAIZE REPRODUCTION

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Arbuscular mycorrhizal fungi (AMF) are important symbionts of terrestrial plants, with an evolutionary history dating back more than 450 million years ago. AMF provide a benefit to the host plant by enhancing nutrient and water acquisition in exchange for carbon and sugars made via photosynthesis. These interactions, manifested through AMF hyphal colonization of soil, are vital not only for the success of agronomically important crops but for soil aggregate formation and carbon sequestration, which contribute to overall soil and crop health. Our prior research shows an inverse relationship between nitrogen fertilization rate and AMF extramatrical biomass in maize that is expressed most strongly at reproduction. This relationship was exacerbated when soybean was the prior crop leading to the hypothesis that prior N fertilization history may also have a legacy effect with respect to N mineralization during the current maize growing season. Addressing how and why this may occur is crucial for understanding how the evolutionary relationship between maize and AMF is impacted by management decisions. In collaboration with an ongoing, long-term field study conducted in Ontario, Canada, we are investigating the impact of nitrogen fertilization on soil microbial biomass and community structure in continuous maize cropping systems where constant and reciprocal N rates have been applied over five-year intervals. This poster will contrast the response of soil AMF biomass to saprophytic fungal and bacterial biomass at maize reproduction to shifting N fertilization regimes¹ and discuss implications for N management in maize cropping systems. Future work will contextualize differences in microbial biomass with

broader ecological processes by exploring taxonomic diversity through amplicon sequencing of root and soil communities.

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POTENTIAL OF THE CO-INOCULATION RHIZOBIUM-BRADYRHIZOBIUM TO IMPROVE THE PRODUCTIVITY OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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Common bean (*Phaseolus vulgaris* L.) nodulates inefficiently with *Bradyrhizobium*. Nevertheless, some strains of this genus enhance the nodulation and growth of this crop when co-inoculated with efficient *Rhizobium* strains under controlled conditions. Based on this finding, we aimed to evaluate the performance of this type of co-inoculation in the field. Two experiments were carried out in Rio de Janeiro, Brazil, during the years of 2017 and 2018. The first experiment evaluated the performance of strains 29 w and BR 85 co-inoculated with *Rhizobium tropici* CIAT 899 following two methods of inoculation (seed and seed + in-furrow). All strains were inoculated at the dose of 1.2×10^6 CFU seed⁻¹, and the in-furrow application was carried out with a dose of $1,08 \times 10^8$ CFU m⁻¹. The second experiment evaluated the co-inoculation of strain BR 85 at doses ranging from 1.5×10^5 to 1.2×10^6 CFU seed⁻¹. In the first experiment, the gain in productivity was greater when the co-inoculation was carried out only on the seeds rather than on seeds + in-furrow. Seed inoculation of BR 85 + CIAT 899 enhanced grain yield by 20% when compared with the inoculation of CIAT 899 only. This response was associated with increased nodulation, plant biomass, and number of pods. The second experiment showed improved nodulation and root biomass as the dose of *Bradyrhizobium* in the inoculant increased, but no significant differences in grain yield were observed. These results show that the *Rhizobium-Bradyrhizobium* co-inoculation has the potential to enhance the growth and grain yield of common bean, although adjustments are needed to ensure its effects in field conditions.

P.137 (will also be presented as poster pitch)

DECIPHERING THE MODE OF ACTION OF BIOSTIMULANT CANDIDATES: A CASE STUDY ON PHOSPHATE-SOLUBILIZING BACTERIA

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The mode of action of a biostimulant refers to its agricultural functions and the underlying physiological and cellular processes. It has to be clearly defined in order to optimize the field application of such products and the related efficacy. Here we present a case study on microbial biostimulants with a focus on phosphorus (P) nutrition, i.e. phosphate-solubilizing bacteria. P deficiency is a common plant nutrition problem in soils due to the formation of poorly soluble P complexes with (i) calcium or (ii) aluminium and iron in alkaline or acidic soils respectively. Rhizobacteria may help enhance root foraging activities and P uptake while improving P

availability to plants. In this work, we studied how such bacteria can modulate root plasticity, i.e. the ability of root development to respond to fluctuating P availabilities.

Brachypodium distachyon (L.) P. Beauv. cv Bd21 plants were cultivated in Magenta® boxes in gravel culture with a modified Hoagland solution containing 25 µM soluble P, 25 µM soluble P + 1 g/l Ca₃(PO₄)₂, 25 µM soluble P + 1 g/l hydroxyapatite or 1000 µM soluble P. The plantlets were inoculated with five selected PSB strains separately, by dipping in a 10⁸ CFU/ml bacterial suspension. The biomass production and allocation were recorded after 28 days of interaction and the root system architecture parameters were measured and analysed using the SmartRoot and IJ_Rhizo softwares combined to the archiDART 3.0 R package.

The plant response to P supply and bacterial inoculation was characterized based on biomass accumulation, root development and the corresponding allometric trajectory analysis. A persistent homology analysis was also performed to compare the morphology of the root systems grown under contrasted P supplies with or without phosphate-solubilizing bacteria. Those results are discussed in the context of P use efficiency and root plasticity assessment in response to environmental factors.

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INSIDE THE TRIPARTITE SEED-ENDOPHYTE-ENVIRONMENT INTERACTION BY COMBINING FTIR SPECTROSCOPY AND SEM

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FTIR spectroscopy hyperspectral and SEM imaging tools were introduced to assess the tripartite wheat kernel-fungal endophyte-environment interaction. In this study, it was confirmed that wheat drought resistance highly depends on coleorhiza-endophyte interaction mechanisms. Composition of coleorhizae of *Triticum durum* was investigated under ambient and drought stress conditions. The OH-stretch IR absorption spectrum suggests that the water-deficit was possibly improved or moderated by kernel's endophytic partner. The fungal endosymbiont direct contact with kernel during germination had highest effect on both lipid and protein (Amide I and II) groups, indicating a potentially increased stress resistance in inoculated kernel. Compared to the indirect kernel-fungus interaction and to untreated kernels (control), direct interaction produced highest effect on lipids. Among treatments, the fingerprint region (1800– 800 cm⁻¹) and SEM images highlighted an important shift in glucose oligosaccharides, most likely linked to coleorhiza-polymer layer disappearance. Acquired differentiation in coleorhiza composition of *T. durum*, between ambient and drought conditions, suggests that FTIR spectroscopy when combined with SEM imaging could be a promising tool for studying endosymbiont-plant interactions within a changing environment.

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POLARITY OF CHLOROFORM ELUENT CRITICAL TO QUANTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGAL BIOMASS IN SOIL USING THE NEUTRAL LIPID FATTY ACID BIOMARKER C16:1CIS11

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Arbuscular mycorrhizal fungi (AMF) are important mutualists forming root associations with the majority of terrestrial plants. In exchange for plant carbon, AMF produce extraradical hyphae that explore the soil and increase uptake of water, phosphorous, nitrogen, and other micronutrients. Quantification of this association, both within the root and soil, has been challenging, traditionally relying on microscopic staining of AMF structures within the roots or hyphae in the soil. Recently, the fatty acid biomarker C16:1*cis*11 has become the mainstay for quantification of AMF biomass within the soil. This biomarker is found within all three lipid classes separated by silica gel chromatography: neutral, glyco- and phospholipids; with the neutral lipid fraction having by far the greatest concentration of C16:1*cis*11 and thereby became the standard for measuring AMF biomass in soils. The neutral lipids are eluted from silica gel with chloroform; however, commercially available chloroform is stabilized with varying amounts of ethanol which influences its chromatographic properties. We found as the amount of ethanol used to stabilize the chloroform increased from 0 to 2.0%, so did the amount of C16:1*cis*11 present in the chloroform extracts, the remainder eluted by acetone in the glycolipid fraction. At ethanol contents above 1.0%, it appears that glycolipids are being extracted along with neutral lipids in the chloroform. Thus, quantification of AMF biomass using C16:1*cis*11 found in neutral lipids is highly dependent on the chloroform used and how it is stabilized. We propose combining the neutral and glycolipid fractions to avoid this uncertainty, or perform a direct hydrolysis of all ester-linked fatty acids (EL-FAMES) *in-situ* using a mild alkaline hydrolysis procedure. A third alternative would be to add a known percentage of ethanol to amylene-stabilized chloroform (non-polar) where the separation between neutral and glycolipid C16:1*cis*11 is optimized.

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EFFECT OF DIFFERENT PROMOTING PLANT GROWTH BACTERIA ON THE REDUCTION OF NEMATODE INFECTION IN TOMATO PLANTS

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Root-knot nematode *Meloidogyne* spp. is one of the most important plant-parasitic nematodes causing significant yield losses in crops worldwide. Recent work is being directed to biological control with microorganisms, including Plant Growth Promoting (PGP) bacteria. The aim of this work was to evaluate the effect of four PGPR strains isolated from potato rhizosphere (Bac15Mb and AZO16M2), and rhizosphere (TRFLT8) and phyllosphere (CRRFLT7) coffee plants on the agronomic parameters of tomato plants (*Solanum lycopersicum* var. "Rio Grande") at greenhouse conditions. For this purpose, plants were inoculated with each

bacteria isolate (10^8 CFU/ml) and 650 eggs and freshly hatched nematodes. The experiment consisted of four treatments and controls with only nematodes (CN) and without nematodes (SN); each with three replicates. Plants were contained in 1 kg pots filled with sterilized sand and vermiculite (2/1) and maintained at $30 \pm 5^\circ\text{C}$. After 25 days of nematode inoculation, treatments with Ba15Mb, AZO16M2 and CRRFLT7 showed a significantly high fresh aerial part weight ($p \leq 0.05$) with respect to the controls, also these isolates performed the significantly high dry aerial part weight with respect to the control with nematodes. Treatments with Ba15Mb and AZO16M2 showed the high significant values of height of plants and fresh root weight with respect to both controls and the high values of number of leaflets with no statistically significant difference. After 55 days, the treatments with CRRFLT7 showed the high value of fresh and dry weight of aerial part and number of leaflets with significant different respect to the controls. Also, the treatment with Bac15Mb showed the high values of dry root weight and height of plant with statistically significant difference. The results obtained suggest that strains CRRFLT7, Ba15Mb and AZO16M2 could be used to improve the response of the plant against the infection of *Meloidogyne*. Acknowledge 177-FONDECYT 2015, 007-PNIA 2016, Investigation Junior-UNAML 2018

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EVALUATION OF DIFFERENT TEMPERATURES IN THE PRESERVATION OF THE ACTIVITY OF ANTIFUNGAL COMPOUNDS PRODUCED BY *BACILLUS* ICBAC02.1 TO CONTROL *RHIZOCTONIA SOLANI*

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The common bean is a legume of economic, cultural and nutritional importance. The use of microorganisms that have the ability to biocontrol plant diseases represent a sustainable alternative in crop management. There are number of preservation techniques mainly temperature use to maintain the activity of antifungal compounds. The aim of this work was to evaluated different temperatures to preserve the activity of antifungal compounds present on Cell-Free supernatant and broth fermentation in order to control *R. solani* at greenhouse level. Antifungal compounds, consisted in hydrolytic enzymes like celluloses, proteases and amylases, as well, antibiotic peptides and siderophores, were produced by culturing *Bacillus* ICBac02.1 on bioreactor conditions. Four treatments were considered: 4°C , -20°C , dried freezer and ambient temperature. Conditions were maintained during one month. It was used common bean cv Canario Centenario plants inoculated with one strain of *Rhizobium* at beginning of the assay. The Treatments were inoculated at the base of 1-week-old plants before infecting the plants with *R. solani*. The results showed that treatment of dried freezer significantly reduce the incidence of the disease by 50% after ten days of evaluation, due to the action of antibiotic metabolites and hydrolytic enzymes. Besides, this treatment present highest value in height (15.1 cm) and fresh (10.2 g) and dry weight (1.4 g) of aerial part with significant differences compared to chemical and pathogen controls. The use of antifungal compounds produced by the culture of *Bacillus* ICBac02.1 preserved by dried freezer represent a promising technology for phytopathogens control and crop management.

Acknowledgement: INNOVATE PERÚ

P.142

ISOLATION AND BIODIVERSITY OF HEAVY METAL TOLERANT ENDOPHYTIC BACTERIA FROM HALOTOLERANT PLANT SPECIES LOCATED IN COASTAL AREAS OF KARACHI, PAKISTAN

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OBJECTIVE: We isolated and identified endophytic bacteria from halotolerant plants collected from coastal areas of Karachi, Pakistan and investigated their heavy-metal tolerance and plant growth promoting potential.

METHODS: In total 57 strains were obtained from 3 halotolerant plants and 29 representative isolates were selected to detect their tolerance against NaCl and heavy metals of Cu²⁺, Pb²⁺, Cd²⁺, Zn²⁺, Hg²⁺; plant growth promoting index of nitrogen fixation, phosphate solubilization, indoleacetic acid (IAA) production and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase.

RESULTS: Most of the isolates could grow under high consistency of Cu²⁺, Hg²⁺ and Pb²⁺. Of the bacteria 39.6% had the ability of nitrogen fixation, 27.9% of phosphate solubilization, 75.7% of IAA production and 45.7% of ACC deaminase activity. The results of 16S rRNA sequencing show that they belonged to the genera of Bacillus, Halobacillus, Oceanobacillus, Exiguobacterium, Serratia, Brevundimonas, Vibrio and Staphylococcus. Among them, strains IP67 and IP71 were potential novel species.

CONCLUSION: The halotolerant plants located in the coastal areas contain a variety of endophytic bacteria as well as the source of novel taxa. Some of them had the ability of plant growth promotion and high resistance against heavy-metal Cu²⁺ and Pb²⁺ and Hg²⁺.

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RHIZOBACTERIA MEDIATED CHANGES IN SOIL PHYSICAL AND HYDROLOGICAL PROPERTIES

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Large communities of microbes are associated with plant roots in the rhizosphere, which is a critical interface supporting the exchange of water and nutrients between plants and their associated soil environment. The diverse communities of rhizobacteria (or soil microbiome) mediate plant-soil feedback through a multitude of interactions including those that contribute to plant abiotic stresses. For example, enhancement of plant drought stress tolerance by plant growth promoting rhizobacteria (PGPR) has been increasingly documented in the literature, however, investigations to date have been largely focused on PGPR-root/plant interactions and related plant responses to PGPR activities that induce drought tolerance. Comparatively, much less is known about PGPR's role in mediating physiochemical and hydrological changes in the rhizospheric soil that may also impact plant drought stress tolerance. Using UD1022, a *B. subtilis* strain, as a model bacterium, we demonstrated via soil water characteristic measurements that UD1022-treated soil samples retained more water and had lower hydraulic conductivity than its controls. In addition, we investigated the effects of UD1022 on soil water evaporation via combined neutron radiography, neutron tomography, and X-ray tomography imaging techniques. Neutron radiography images showed greater water retention in UD1022-treated soil

samples than their controls due to reduced water evaporation. Combined neutron and X-ray tomography 3D images revealed that water distribution in UD1022-treated soil samples was heterogeneous, i.e., there were more disconnected water pockets compared with the controls where water was distributed more uniformly. Water evaporation from the disconnected water pockets was slower than more uniformly distributed water film. Our study provides pore-scale mechanistic explanation for the reduced evaporation rate for UD1022-treated soil samples. Enhanced water retention in soil and reduced evaporation by UD1022 treatment is likely due to the production of extracellular polymeric substances (EPS) by UD1022 that changed soil pore structure, which was visualized by scanning electron microscopy (SEM).

P.144 (will not be presented)

EFFECT OF LONG-TERM APPLICATION OF COMPOST AND BIOCHAR ON COWPEA RHIZOSPHERE MICROBIAL COMMUNITIES AND PHYSICOCHEMICAL PROPERTIES OF SOIL

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Arid and semi-arid regions like UAE face various obstacles in boosting agricultural production and food security. One of the major hindrances to healthy growth of crops in these regions is the salinity of the nutrient-poor soil. As part of mitigation strategies, organic amendments such as biochar can be added to sandy soil to improve soil nutrients and fertility. Biochar is a charcoal produced by pyrolysis of agricultural mass. Biochar can augment soil properties for agricultural use by improving its physical and chemical properties to retain more water and nutrients. Our study compared the effect of long-term application of biochar and compost on physiochemical properties and cowpea rhizosphere microbial community structure. The samples were collected from a plot of cowpea designated for field experiments. The sample group comprised of control sample, compost-amended soil for one year (C1), compost-amended soil for three years (C3), biochar-amended soil for one year (BC1), biochar-amended soil for three years (BC3). For evaluating the long-term application of biochar on soil, water retention, pH, electrical conductivity (EC) were analyzed and compared with samples treated with the long-term compost application. Molecular techniques and next generation sequencing analysis was used to investigate the effect of biochar on soil microbial community structure relative abundance and diversity. The results indicated that the long-term application of biochar has positive impact on the soil fertility and plant growth. Plants grown in long-term biochar enhanced soil samples accumulated more nutrients than the short-term amended soil samples. Furthermore, microbial groups were found to be more diverse and abundant in long-term biochar treated soil samples. In conclusion, long-term application of biochar significantly improved sandy soil physiochemical and microbial characteristics.

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ACCLIMATION AND SEASONAL GROWTH OF PERENNIAL GRASSES: INSIGHT INTO ROOT APEX CELLULAR DEVELOPMENT AND PLASMA MEMBRANE PHYSIOLOGY

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Plant roots are especially sensitive to environmental cues. The survival of perennial grasses under conditions of seasonality greatly depends on their capacity to modulate their growth, and to acquire appropriate adjustments prior to wintering. A comparison of a hardening temperature (+8/+2°C) response was studied in the root apical meristem (RAM) of two closely related species differing in their winter hardiness: namely highly resistant *Festuca pratensis* and less hardy ×*Festulolium braunii* hybrid, where *F. pratensis* is the parental component of the hybrid. Differences in root apex cellular development were apparent by the explicit enhancement of the RAM in *Fl. braunii*, along with the sharp cell width increment compared with that in *F. pratensis*. These findings suggest a different pattern of plant growth in autumn / early winter period, namely, ×*Fl. braunii* expands its RAM and keeps growing, while *F. pratensis* declines cellular proliferation in the RAM, and halts its vegetative growth. As ×*Fl. braunii* roots stay physiologically active in the late season, sudden lowering of the temperature can be damaging to plants, which explains their lower winter survival.

The plasma membrane is considered to be the primary site of the cell physiology adjustments induced by environmental stress. Therefore, the question that arises is: could the functional state of plasmalemma be related to plant freezing tolerance? Changes in root cell plasmalemma functional activity were manifested differently in response to the low temperature in freezing-tolerant and non-tolerant *F. pratensis* at hardening/stressogenic (+4 - +2 °C) and at freezing (-8 °C) temperatures. In addition, a specific seasonal fluctuation of K⁺Mg²⁺-ATPase activity in the roots of *F. pratensis* was determined under conditions of a three-year field experiment. These results lead to the assumption that a specific proton transport regulation via coupled changes in the hydrolyzed ATP/transported proton ratio could be a property of freezing-tolerant plants.

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METABOLITE PROFILING OF SOYBEAN ROOT EXUDATES UNDER DIFFERENT POTASSIUM STATUS

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Root exudates play a major role in the mobilization of nutrient in the rhizosphere. Comprehensive effect of potassium (K) status on root exudates metabolites of soybean is not known. Two soybean cultivars (Satonohohoemi as low-K-tolerant and Tachinagaha as low-K-sensitive) were grown hydroponically with 6 (K6), 60 (K60) and 120 (K120) mg K L⁻¹ for 7 and 14 days after transplanting (DAT). Root exudates were collected and metabolites were determined using capillary electrophoresis/time-of-flight mass spectrometry (CE-TOF MS).

Root K concentration of Satonohohoemi and Tachinagaha was lower at K6 than at K60 and K120 for 7 and 14 DAT. The number of metabolites detected in the root exudates of Satonohohoemi and Tachinagaha was 43 and 39, respectively. Seventeen and 7 metabolites increased, 17 and 25 metabolites decreased in Satonohohoemi at 7 and 14 DAT under K6 level, respectively. Nine and 11 metabolites increased, 19 and 20 metabolites decreased in Tachinagaha at 7 and 14 DAT under K6 level, respectively. Fifteen and 18 metabolites increased, 14 and 14 metabolites decreased in Satonohohoemi at 7 and 14 DAT under K120 level, respectively. Fifteen and 7 metabolites increased, 18 and 20 metabolites decreased in Tachinagaha at 7 and 14 DAT under K120 level, respectively. The score plot of the principal component analysis (PCA) showed a clear grouping of the root exudates metabolites under three potassium level and two times of sampling. The first PCA showed 97.4% in Satonohohoemi and 95.8% in Tachinagaha. The second PCA showed 1.7% in Satonohohoemi and 2.6% in Tachinagaha. We will discuss the role of metabolites in root exudates in potassium nutrient.

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FUNCTIONAL DIVERSITY OF THE BACTERIAL COMMUNITY AND MICROBIAL BIOMASS IN FLOODPLAIN FOREST SOILS

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The floodplain forests of southern Moravia were regularly flooded in the past, usually at the end of winter. However, regulation of the rivers and establishment of dams and reservoirs in the 1970s has greatly reduced the incidence of flooding. As a consequence, the conditions in the floodplain sites have become relatively dry, and instead of plant species that are adapted to temporal anoxia, the vegetation now consists of more opportunistic species.

This study investigated the impact of the reduction in flooding on microbial biomass and functional diversity of the bacterial community in different soil layers in a floodplain forest.

A 110-year-old floodplain forest composed of typical hardwood species and located in Lanžhot (Czech Republic) was selected for study. In the past 40 years, flooding has occurred only rarely in this site and only for short periods. Soil samples (Fluvisol) were collected at depths of 0-5, 5-15 and 15-30 cm in two plots. Microbial biomass C was determined by the fumigation-extraction method, and microbial community-level physiological profiles (CLPPs) were constructed using Biolog EcoPlates™. The physico-chemical properties of all samples were also characterized.

The soil organic matter content and microbial biomass decreased sharply with depth. Average well colour development (AWCD), community metabolic diversity and Shannon and evenness indices were generally much higher in surface samples than in the two deepest layers (especially for AWCD and the Shannon index), in which the values were similar.

The reduction in fresh organic matter and nutrients that previously entered the soil profile through flooding and sedimentation cycles has led to a change in the soil organic matter content (and all associated properties),

from the typical vertical distribution of floodplain soils to that characteristic of terrestrial soils. The change has occurred faster for the organic matter and microbial biomass than for properties related to functional biodiversity.

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METABOLOME ANALYSIS OF ROOT EXUDATES OF WILD AND CULTIVATED RICE UNDER PHOSPHORUS DEFICIENT CONDITION

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Plants release various metabolites from roots and root exudates contribute to differences in stress tolerance among plant species. The purpose of this study was to examine the differences of metabolites in root exudate among wild and cultivated rice. Wild rice (*Oryza rufipogon* (W1294), *O. rufipogon* (W1945) and *O. brachyantha* (W1711)) and cultivated rice (*O. sativa* cv. Akamai and *O. sativa* cv. Koshihikari)) were grown in soils fertilized with 0 g P kg⁻¹ (-P) or 4.8 g P kg⁻¹ (+P). Root exudates were collected at 13 and 20 days after transplanting (DAT) and their metabolites were determined by capillary electrophoresis/time-of-flight mass spectrometry (CE-TOF MS). One hundred fifty-nine to 238 metabolites were detected in the root exudates and 104 metabolites were common to all species. The number of metabolites increased in -P and +P at 13 DAT were 8 and 8 in Akamai, 42 and 12 in Koshihikari, 19 and 6 in *O. rufipogon* (W1294), 6 and 22 in *O. rufipogon* (W1945), and 18 and 2 in *O. brachyantha* (W1711), respectively. The number of metabolites increased in -P and +P at 20 DAT were 2 and 51 in Akamai, 2 and 14 in Koshihikari, 8 and 26 in *O. rufipogon* (W1294), 4 and 41 in *O. rufipogon* (W1945), and 9 and 5 in *O. brachyantha* (W1711), respectively. Twenty amino acids were increased in -P of cultivated rice, but less than 5 amino acids were increased in -P of wild rice. We will discuss the relationship between metabolites detected in root exudates and rhizosphere microbiome.

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TETRAPLOID EXHIBITS MORE TOLERANT TO SALINITY THAN DIPLOID IN SUGAR BEET (BETA VULGARIS L.) SEEDLINGS

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Salinity is a major abiotic stress factor limiting plant growth and crop yield worldwide. The objective of the present study was to compare differences of salinity tolerance between diploid and tetraploid seedlings of sugar beet (*Beta vulgaris* L.) exposed to different concentrations (0, 50, 100, 200 and 300 mM) of NaCl. The results showed that shoot fresh weight (FW) and dry weight (DW) in tetraploid were remarkably higher than those in diploid when subjected to different concentrations of NaCl. At 200 and 300 mM NaCl, tetraploid obviously accumulated less Na⁺ in its shoots and roots compared with diploid. However, there were no differences in K⁺ accumulation between tetraploid and diploid under salinity stress. Our results also showed

tetraploid displayed a lower Na^+/K^+ ratio and a greater selective capacity for K^+ and Na^+ than diploid when exposed to high-salt stress. Furthermore, it was observed that tetraploid possessed a lower net Na^+ uptake rate and a higher net K^+ uptake rate compared with diploid under high-salt condition. We also found that *BvHKT1;1*, *BvNHX1*, *BvSKOR* and *BvSOS1* were induced by 50 mM NaCl, and their transcript abundances in tetraploid were relatively higher than those in diploid. Additionally, soluble sugars contents were obviously higher in tetraploid than in diploid when exposed to 100, 200 and 300 mM NaCl. Taken together, these results suggested that tetraploid exhibited more tolerant to salinity stress than diploid in sugar beet by accumulating less Na^+ and more soluble sugars, and by maintaining lower Na^+/K^+ ratio and greater capacity of selective absorption for K^+ over Na^+ . The results of this study provide insights into physiological and molecular consequences of polyploidization in sugar beet, which might have practical implications for genetic improvement of salt tolerance in sugar beet and other crops.

P.150 (will not be presented)

RHIZOSPHERE MICROORGANISMS AND PLANT STRESS-RESISTANCE IN ANTARCTICA

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Despite being one of the harshest environments on Earth, Antarctica is home to two vascular plants, *Colobanthus quitensis* and *Deschampsia antarctica*. Both of these plants have been shown to partially rely on their rhizosphere microorganisms to withstand extreme conditions such as low available water and high salinity. Moreover, differences in the distribution of the two plants suggest a difference in stress-resistance capacities, with *D. antarctica* growing in more extreme areas than *C. quitensis*, and *C. quitensis* being found in the most exposed area only when associated with *D. antarctica*. Using amplicon sequencing, we studied the taxonomic compositions of bulk soil, root-associated soil and rhizospheric soil of the two plants growing independently and in association. We find that, for both bacteria and fungi, the sample location is a strong determinant of rhizosphere and root-associated microbiome composition, and that there is no clear separation between samples taken from bulk soil, root-associated soil and rhizosphere when looking at overall taxonomic composition. However, we found differences in the overall fungi composition of *C. quitensis* rhizosphere and root-associated soil compared to *D. antarctica*, and *C. quitensis* in association with *D. antarctica*. We also find 15 bacterial amplicon sequence variants and five fungal amplicon sequence variants that show species-specific abundance. For both fungi and bacteria, when differences are observed between plant species, *D. antarctica* and *C. quitensis* in association with *D. antarctica* are found to be very similar. This suggests that when growing in association to *D. antarctica*, *C. quitensis* does adopt microorganisms from *D. antarctica*, which could allow it to withstand more extreme conditions. Our results also suggest that microorganisms that are important to plant stress-resistance might not be among the most abundant taxa in the rhizosphere.

P.151**CONTRIBUTIONS OF THE ROOT MICROBIOME TO CROP ABIOTIC STRESS TOLERANCE IN SOIL RECEIVING ORGANIC AMENDMENTS****M McMillan**¹, C Kallenbach¹, J Whalen¹¹*McGill University, Montreal, Qc, Canada*

Root microbiomes have the potential to alleviate crop heat and drought stress by reducing plant stress ethylene through the production of 1-aminocyclopropane-1-carboxylate deaminase. Abiotic stress tolerance is further expected to improve when crops and their associated root microbiomes have ample soil nutrient supply. Biosolids are a nitrogen-rich organic amendment that have been shown to lead to improved soil health and crop yield. They are also rich in soluble carbon and contains their own microbes which could potentially stimulate the root microbiome more than fertilizer alone. The objective of this study is to determine how soybeans and their associated rhizobacteria respond to heat and drought stress when fertilized with biosolids. Greenhouse grown soybeans were subjected to one of four stress treatments (no stress, heat stress, drought stress or heat and drought) and received either biosolids or control fertilizer. Heat stress was induced at 9 weeks by increasing diurnal air temperature from 25 °C to 35°C. Drought stress was induced by reducing soil moisture from 50% soil water holding capacity to 5-8%. After 10 days of stress, plant-soil headspace ethylene is measured by GC-FID and plant biomass and lengths are measured with WinRhizo. The functional diversity of plant growth promoting bacteria possessing 1-aminocyclopropane-1-carboxylate deaminase genes is examined via qPCR of 1-aminocyclopropane-1-carboxylate deaminase structural genes (*acdS*) and by direct enzyme quantification. The impacts of organic fertilizers on root microbiome functional diversity and production of plant ethylene under heat and drought stress will be presented.

P.152**MICROBIAL BIOMASS AND ENZYMATIC ACTIVITY IN THE RHIZOSPHERE OF ORGANICALLY AND CONVENTIONALLY MANAGED ORCHARD CROPS CULTIVATED IN ARID SOIL****KD ALOTAIBI**¹, M Arcand², N Ziadi³¹*King Saud University, Riyadh, Saudi Arabia*²*University of Saskatchewan, Saskatoon/SK, Canada*³*Agriculture and Agri-Food Canada, Quebec/QC, Canada*

Organic agriculture is perceived as an alternative agricultural system that is assumed to be less harmful to the environment and provide healthy and nutritious food products. However, whether organic farming practices sustain crop yields or improve soil quality relative to conventional farming is both system and location dependent. Organic farming is an expanding practice in Saudi Arabia, emerging in late 1990s and firstly adopted in tree orchard cropping systems, such as olive and peach. The extent to which the organic agricultural management practices under extreme environment conditions of Saudi Arabia can affect the arid soil quality and biochemical properties is still largely unknown. Therefore, this study aimed at comparing the temporal impact of organic vs. conventional management practices on microbial biomass and enzyme activities in the rhizosphere of olive and peach trees. Rhizosphere soils were collected from organically and conventionally

managed olive and peach orchards located within short distances from each other and representing three plantation periods: 20, 15 and 10 years. The activities of β -glucosidase, N-Acetyl-Glucosaminidase and phosphatase enzymes in addition to microbial biomass carbon and nitrogen will be examined and their results will be presented.

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METATRANSCRIPTOMIC RESPONSES OF THE WHEAT HOLOBIONT TO DECREASING SOIL WATER CONTENT

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Wheat productivity in western Canada is constrained by increasing and recurring drought, with total yields at the national level expected to fall by 11.5% as compared to 2017. Conventional approaches of wheat breeding can partly solve this problem, but the time taken to generate new and successful varieties cannot match the rate of declining wheat yields. Alternatively, a promising approach is to manipulate the plant-associated microbiota resulting in rapid improvement in wheat resistance to water stress. However, the combined and coordinated responses of the wheat and its microbiota (the wheat holobiont) to water stress are poorly understood. The principal aim of this experiment was to determine the metatranscriptomic response of the wheat holobiont to water stress in the field. Wheat was grown in the field and received 100%, 75%, 50% or 25% of the natural precipitation using rain exclusion shelters. RNA was extracted from the rhizosphere soil and roots samples collected in July, at the peak of the growing season. Root eukaryotic and rhizosphere prokaryotic mRNA libraries were constructed independently and sequenced using Illumina HiSeq. The preliminary results show that both abundances and activities of the prokaryotic as well as eukaryotic components are affected by the level of precipitation in the wheat rhizosphere soil. Further analysis of the metatranscriptomic data will provide an in-depth characterization of the genes and pathways involved in drought tolerance of the wheat holobiont and allow us to identify which holobiont partners are responding to decreasing soil water content. Our long-term objective is to engineer the wheat holobiont for increased drought resistance and resilience.

P.154

ELEVATED CARBON DIOXIDE INCREASES SOIL ORGANIC CARBON IN RICE PADDIES

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Rising atmospheric carbon dioxide (CO₂) levels contribute to climate warming, but may be positive for agricultural production. High-CO₂-responsive rice cultivars produce more biomass and about 30% more grain when exposed to elevated CO₂. Although high-CO₂-responsive rice will require a greater soil nutrient supply to achieve these outcomes, it should also produce more fine roots and exudates, which could increase soil organic

carbon levels and macroaggregate formation. The objective of this study was to determine how elevated CO₂ and nitrogen addition affected soil organic carbon pools and aggregates in rice paddies planted with high-CO₂-responsive and low-CO₂-responsive cultivars. Paddy soils receiving elevated CO₂ from free air CO₂ enrichment had greater soil organic carbon and readily-oxidizable organic carbon content, but elevated nitrogen (225 kg ha⁻¹) alone reduced the soil organic carbon and readily-oxidizable organic carbon, relative to the untreated control. More large macroaggregates with greater mean weight diameter were found in the elevated CO₂ plus nitrogen treatment than the untreated control, regardless of the rice cultivar. Aggregate formation differed under high-CO₂-responsive and low-CO₂-responsive cultivars in the presence of elevated CO₂ alone or nitrogen addition alone. This suggests that root-associated processes together with soil nutrient supply influences the aggregate formation in rice paddies, and this possibility will be discussed.

P.155 (will not be presented)

NOVEL ENDOPHYTIC TAXA WITHIN THE PLEOSPORALES AND THEIR INFLUENCE ON PLANT GROWTH

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Grasses in desert ecosystems experience stressful abiotic conditions including drought and heat and are abundantly colonized by dark septate endophytic fungi. These fungi occur worldwide and include species from multiple orders of the phylum Ascomycota including Pleosporales. Prior work has suggested these endophytes are critical for plant survival and adaptation to drought. We described and characterized a novel group of isolates; further experiments will evaluate their effects on host plants under drought. Fungi were isolated from roots of three native grasses distributed across 18 sites spanning replicated latitudinal gradients in the US. Isolates were characterized using ITS rRNA and LSU rRNA gene sequencing. We compared sequences against the NCBI database and performed phylogenetic analyses using MEGA7. Cultures were also morphologically characterized using different media and growth conditions. Colonies on MEA grew >35 mm diameter in 14 days, presented very diffuse, aerial white mycelium and appeared white, brown, or beige on the underside of the colony. Some colonies on PDA were brown, olivaceous, or beige and stained the media. Isolates had dark septate melanized hyphae when mycelium was older than >14 days. Chlamydospores were found in the mycelium, terminal or intercalary, sometimes solitary; smooth cell wall and oil-droplets characterized the dark hyphae. These novel isolates likely belong to the family Montagnulaceae and are likely closely related to the genera *Kalmusia* and *Didymocrea*, representing a candidate new specie. However, additional genes will be sequenced to confirm its placement relative to known taxa.

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EFFECTS OF HALOPHILIC BACTERIAL INOCULANTS ON THE DEVELOPMENT OF A SALT-SENSITIVE PLANT

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The use of halophilic or halotolerant bacteria as plant growth promoters is one of the strategies recommended for the mitigation of salt stress and most commonly, halotolerant bacteria isolated from the rhizosphere of halophytes are the used inoculum.

The objective of this work was to evaluate the effect of halophilic bacteria, isolated from an active salt pan, on *Lactuca sativa*, used as a model crop glycophyte, exposed to saline stress.

Strains of *Bacillus*, *Halobacillus*, *Idiomarina* and *Marinobacter*, were analysed for favourable traits in terms of root colonization and plant growth promotion potential (extracellular enzymes, phosphate solubilization, production of 1-aminocyclopropane-1-carboxylate deaminase and indoleacetic acid). *H. locisalis* and *I. seosinesis*, considered as the most interesting in terms of plant growth promoting traits, were tested individually or combined, as inoculum in lettuce seeds. A factorial experimental design was applied to test the effect of inoculation and salinity of the irrigation water (0 or 10 PSU) on the efficiency of seed germination and plant growth.

Germination efficiency was strongly affected by salinity and no significant attenuation effect could be attributed to inoculation. The germination efficiency was lower at salinity 10 than at salinity 0 and the weight of the plants, as well as water content, was even lower in the plants inoculated with the consortium of isolates than in the non-inoculated controls.

However, when used separately, *H. locisalis* or *I. seosinesis* caused an increase in leaf size in plants cultivated in non-saline conditions, in relation to the non-inoculated control.

Although no evidence of attenuation of saline stress by inoculation was detected, inoculation with individual halophilic strains affected morphological traits of plants grown in non-saline conditions, indicating the these halophilic bacteria, although not isolated from the rhizosphere of plants may represent some potential as growth modulators for plants of agricultural interest.

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THE BACTERIAL MICROBIOMES OF FIELD CROPS IN SASKATCHEWAN, CANADA

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Research on plant microbiomes is important to elucidate key functions of bacteria inhabiting crops thus contributing to the improvement of agricultural production. This study used DNA high-throughput sequencing to assess bacterial communities associated with the rhizosphere and root interior of canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* L.) and field pea (*Pisum sativum* L.) grown on agricultural sites in Saskatchewan. In addition, the influence of plant growth stages on bacterial microbiomes of wheat and canola was assessed in a growth chamber study. Assessment of field grown crops revealed rhizosphere bacteria profiles were unique among crop species and sampling locations. Individual crops associated with distinct root

endophytic bacterial communities, reaffirming that root endophytes are a subset of the rhizosphere microbiome. The genera *Pseudomonas* and *Stenotrophomonas* were predominant in the rhizosphere and root interior of all crops, suggesting a generalist distribution of these bacteria. However, other genera including *Arthrobacter*, *Streptomyces*, *Rhizobium*, *Variovorax* and *Xanthomonas* also were abundant in the root interior of specific crops. Bacterial communities colonizing aboveground plant organs varied greatly among crop species, soils and plant organs and consisted mainly of *Corynebacterium*, *Pseudomonas*, and unclassified Enterobacteriaceae. In our study, the relative abundance of specific bacterial groups in the rhizosphere correlated significantly with soil pH, silt and organic matter contents. There was, however, no correlation between analyzed soil properties and most abundant endophytic bacterial genera. These results indicate that soil characteristics may not influence bacterial communities within plant roots. Moreover, the bacterial microbiome in rhizosphere, root and stem of wheat and canola was influenced by plant growth stages and this effect was mainly crop and organ specific.

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IMPACT OF INDIVIDUAL AND COMBINED ABIOTIC STRESSES ON PLANT GROWTH PROMOTING BACTERIA

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Abiotic stress conditions, such as drought and salinity, are some of the foremost limiting factors for agricultural productivity. The effects of abiotic stresses on plants and plant growth promoting bacteria are typically being studied individually under controlled growth conditions, but under field conditions plants are exposed simultaneously to more than one abiotic stress, such as combination of drought and salinity. Recent studies revealed that bacteria and plants respond to a specific combination of stresses in a non-additive manner, producing complex effects that could not have been predicted from the study of either stress individually. This study was designed to investigate the complexity of individual and combined stresses by measuring different characteristics of four endophytic bacterial strains (32a, 727b, 11e and D7G) related to plant growth promotion and endophytic colonization of tomato plants. These characteristics included stress tolerance, auxin and biofilm production, swimming and swarming motility, endophytic colonization and plant growth promotion. Drought, salinity and combined stress were induced using different concentrations of polyethylene glycol and sodium chloride, alone or in combined mixtures. Bacterial strain 32a was less negatively affected than the other tested strains on auxin and biofilm production under drought and salinity as compared to the combined stress. Swimming and swarming motility of 11e was less negatively affected than the other bacterial strains under individual and combined stress. Endophytic colonization and tomato plant growth promotion of 727b were less negatively affected under combined stress as compared to individual stresses (salinity and drought). Salinity

and drought tolerant plant growth promoting bacteria characterized in this study will be further developed as biofertilizers to mitigate the negative effects of abiotic stresses on crop growth and production.

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FINDING THE NEEDLE IN A HAYSTACK - DEVELOPMENT OF MICROBIAL FERTILIZERS OR PESTICIDES FOR SUSTAINABLE AGRICULTURE

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Plants are holobionts that are associated with complex microbiomes, and many endophytes that reside within plant tissues have plant growth promoting capabilities. To maintain the increasing growth requirements of food and crop production, alternative strategies to chemical fertilizers and fungicides will need to be developed to ensure cost-effective and environmentally sustainable agriculture in the future. The application of plant endophytes as biological fertilizers and pesticides have the potential to positively impact crop yields under a broad range of abiotic (e.g. drought, salinity, heavy metals, nutrient deficiencies) but also biotic stress conditions (e.g. pathogenic bacteria or fungi, or plant herbivores). Bacterial endophytes can promote plant growth through their ability to produce plant growth hormones, fix atmospheric nitrogen, solubilize recalcitrant phosphate or potassium resources, suppress the growth of phytopathogenic fungi, or decrease the levels of the plant growth stress hormone ethylene through their biosynthesis of 1-aminocyclopropane-1-carboxylate. We isolated diazotrophic bacterial endophytes from *Brassica carinata* on nitrogen free agar, and characterized their plant growth promoting characteristics through a diverse set of *in-vitro* assays. We conducted root vigor assays and examined root architectural traits, greenhouse trials and field studies to test the capability of these plant endophytes to promote the growth of soybean, corn or wheat plants under a variety of different stress conditions. There is a high variability in the plant growth response after endophyte inoculation among different plant species but also among different cultivars of one species. We will provide an overview of these results and will correlate the observed plant growth promoting capabilities of different strains to data mining approaches after whole genome sequencing of the different plant endophytes. In addition, we will discuss the reliability with which different screening tools are able to predict plant growth promoting capabilities of endophytes for different crop species under different field conditions.

P.160 (will also be presented as poster pitch)

DOES ROOT MUCILAGE PLAY A ROLE IN SALINITY TOLERANCE?

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Soil salinization is a growing threat to environments across the globe, affecting over 800 million hectares of land spread across 100 countries. This area is estimated to increase as climate models predict a major alteration in rainfall patterns combined with rising sea levels. With the tandem effects of climate change and soil salinization beginning to take hold, a greater understanding of the mechanisms driving plant abiotic stress tolerance is becoming increasingly urgent. Hidden below the soil surface, plant roots are intimately exposed to

the pressure of salt stress. Often, the first line of defense is the production of a high molecular weight mucilage, at a significant energy cost, by the root cap to alter the immediate environment. The cocktail of sugars, proteins, lipids and DNA within the mucilage may combat osmotic stress, as well as trapping salts. This study aims to untangle the complex relationships between mucilage and the root cap under salinity stress. Analysis of root mucilage of an established cereal crop *Zea mays* and a crop becoming significant for the manufacture of gluten-free foods, *Plantago ovata*, via high performance liquid chromatography (HPLC) has revealed changes in sugar profiles driven by increasing salinity concentrations. Additionally, salinity-induced alterations in root cap morphology observed using confocal microscopy may be related to changes in cell wall structure and composition. These results suggest that fine transcriptional control may be orchestrating events in the root cap as a result of salt-stress. Targeted RNA sequencing analyses will be used to map these changes. This work will further illuminate the dark world of plants roots and their ability to withstand the pressures of an ever-changing environment.

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ECTOMYCORRHIZAL EXOENZYMATIC ACTIVITY IS TIGHTLY COUPLED WITH HOST FOLIAR NUTRIENT CONTENT

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Phosphorus deficiencies are increasingly recognized as a major constraint to ecosystem productivity across the temperate rainforests of British Columbia. Temperate forest trees acquire much of their phosphorus through the activity of their ectomycorrhizal associations, which can use extracellular enzymes to access otherwise inaccessible organic P. Through measuring phosphorus-specific exoenzymatic activity of ectomycorrhizal root-tips, along with activities of exoenzymes that degrade lignocellulose or chitin, we set out to evaluate the drivers of exoenzymatic activities along a phosphorus gradient in west coast Douglas-fir forests. Twelve sites across southern Vancouver Island, Canada, were sampled. Inorganic P declined nine-fold from less-weathered, east coast Brunisols to more strongly weathered, west coast Podzols, resulting in the P content of highly-weathered sites being ~ 84% Po. Reductions in foliar P concentrations confirmed P deficiencies over most Podzol sites. We assayed phosphomonoesterase and phosphodiesterase activities on the surfaces of individual ectomycorrhizal root-tips to test the hypothesis that phosphatase activities would increase as soil Po/Pi increased. Activities of laccase, N-acetylglucosaminidase, b-glucosidase, and xylosidase were measured because they can act on plant and fungal cell walls in litter, releasing more nutrients. Phosphatase activity on the mycorrhizoplanes, especially phosphodiesterase, was inversely related to soil inorganic P content, (adj-R²=0.59; P=0.002); the activity increased ~2.5X from less-weathered to more-weathered soils. There was no relationship with organic P. Interestingly, phosphodiesterase activity was negatively related to foliar P concentrations (adj-R²=0.58; P=0.002), and even more tightly coupled, positively, to foliar N:P ratio (adj-R²=0.73; P<0.001), increasing in activity ~4.5X along the gradient. N:P ratio in the foliage was the only soil or foliage variable that explained a significant amount of variation in all six enzyme activities. We conclude that fungal exoenzyme activity in these

P-limited sites was most strongly aligned with host P status in relation to N, rather than with P availability alone.

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THE EFFECT OF COMBINED SALINITY AND LOW ROOT ZONE TEMPERATURE ON TOMATO ROOTSTOCKS

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Stressful environmental conditions are causing major losses to agricultural production worldwide. Among these stresses, salinity is a significant environmental abiotic stress factor and approximately 20% of agricultural lands worldwide are affected by it, mostly arid and desert lands. Furthermore, agricultural crops may be influenced by a combination of abiotic stresses simultaneously. One kind of combination could be salinity and chilling stress: a winter crop abiotic stress factor which can occur in open fields or non-heated greenhouses. Earlier studies have discovered that the combination of different stresses causes a unique response that cannot be determined directly as a response to each of the occurring stresses.

The aim of this research is to study and compare the effect of combined salinity and low root-zone temperature (LRZT) stress on different tomato rootstocks. Furthermore, it aims to explore root structure and function as a mechanism to sustain these stresses. Three tomato (*Solanum lycopersicum* L.) rootstocks, differing in their response to stress, were exposed to two levels of root-zone temperatures (10 and 25 °C) and two levels of salinity (1 and 6 dS m⁻¹), while growing in an aeroponics apparatus. Gas exchange, elemental composition, osmolality levels, shoot and root phenology and anatomy were examined. Initial results show that the combined stress significantly inhibited gaseous exchange and reduced plant height and leaf growth. LRZT reduced maximum root length, root projected area and shoot and root dry mass, with a greater effect on the grafted wild line (20) and on the self-grafted commercial line (Lurka) than on the grafted commercial line (Arnold). Our results suggest that the enhanced tolerance of Arnold to LRZT, is likely linked to a more effective hydraulic behavior and metabolism alteration, which will be further examined. Finally, this research will hopefully provide knowledge for good management of tomato cultivation in semi-arid environments.

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PHYLLOSHERE BACTERIAL MICROBIOTA OF COFFEA ARABICA VAR. CATURRA ROJA USING A METAGENOMIC APPROACH AND THE ANTAGONISTIC POTENTIAL OF CULTURABLE BACTERIA AGAINST COLLETOTRICHUM SP.

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Coffee is one of Peru's main agricultural export. Among *Coffea arabica* varieties grown in Peru, Caturra Roja is known for its excellent cup quality and high productivity; however, it is susceptible to various diseases. Anthracnose caused by *Colletotrichum* spp. is a devastating disease that infects coffee leaves and fruits, diminishing plant productivity. Nowadays, biological control has emerged as an alternative against phytopathogenic fungi with successful results. This study aimed at determining the bacterial microbiota in leaves of Caturra Roja and to obtain antagonistic strains against *Colletotrichum* spp. A targeted metagenome

approach directed at the 16S ribosomal gene was used. Tests for antagonism were performed by the dual culture agar plating method and potential strains were assayed for protease, lipase, chitinase and lecithinase activities. Thirteen phyla were identified, however nine were present at very low abundances (<0.5%). Samples were dominated by Proteobacteria and Actinobacteria with abundances of 63.6% and 29.1%, respectively, followed by Bacteroidetes (4.8%) and Firmicutes (1.9%). Most Proteobacteria belonged to subclass alpha with *Methylobacterium* being especially predominant in the samples (41.8%). *Rhodococcus* represented 5% of the microbiota and the most abundant Actinobacteria while *Flavobacterium* and *Chryseobacterium*, each with abundances of 1.75%, were the most common Bacteroidetes. Individually, each genus of Firmicutes did not represent more than 0.5% of the total diversity. Genera with known antagonistic representatives like *Pseudomonas*, *Bacillus*, *Burkholderia* and *Lysobacter* were present at low to very low abundances, i. e. 2%, 0.2%, 0.01%, 0.01%, respectively. Six out of twenty isolates obtained by culturable approaches showed percentages of inhibition between 28% and 58% against *Colletotrichum* spp., and three displayed protease, chitinase and lipase activities, which could explain their antagonism. Taxonomic affiliation of the identified antagonists is under way and will be presented at the conference. Acknowledgement: 007-2016-INIA-PNIA/UPMSI/IE Project.

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PHYLOGENETIC DIVERSITY OF PSYCHROTOLERANT RHIZOBACTERIA ASSOCIATED TO CHENOPODIUM QUINOA FROM PERUVIAN ANDEAN HIGHLANDS

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Chenopodium quinoa Wild. (or quinoa) is a cereal with high food importance globally due to its nutritional properties. In South America, Peruvian Andean highlands are one of the most important productions zones of native varieties. Adverse environmental conditions of these zones such as low temperatures could affect quinoa growth and development. Root-associated microbial communities are very important in the adaptation of plants to its environments because these interactions could be beneficial to the plants to make them more resistant to different stress factors like low temperatures. There is only a few pieces of information about microorganism communities in interactions with the rhizosphere of quinoa. The aim of this study was to perform a phylogenetic diversity analysis of psychrotolerant native bacteria present in the rhizosphere of Peruvian *C. quinoa*. Fifty-five isolates related to the rhizosphere of quinoa plants were studied. Forty-one of these strains are able to grow at low temperatures (4 and 6°C). The diversity present in these psychrotolerant strains was obtained with a BOX-PCR, detecting 14 different BOX patterns. Phylogenetic analysis was based on the 16S rRNA gene sequence of strains with distinct BOX patterns obtained. It was detected the phyla Actinobacteria (with *Pseudarthrobacter*), Firmicutes (with *Bacillus*) and Proteobacteria, being this last one the most abundant with representative genera of *Pseudomonas*, *Enterobacter*, *Acinetobacter* and *Burkholderia*. More of the most of these genera present cold tolerance species and are adapted to low temperatures. In this context, rhizobacteria that could tolerate environmental stress factors such as low temperatures have a great biotechnological potential for agriculture because they could improve the adaptation of native varieties of quinoa plants in Peruvian highlands soils,

where the climatic stress conditions due to low temperatures are a limiting factor in the agriculture in this region.

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THE SEED MICROBIOME AND ITS CONTRIBUTION TO THE PLANT HOLOBIONT

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The seed microbiome of *Lactuca sativa* (Cos-Romaine lettuce) contains a heritable microbiome. Upon germination, seed endophytes take various routes to fulfill their intended role in the plant life cycle. The first step in determining their role in plant growth is to determine the path they take from seed to various plant compartments. In this study, seeds and germinated seedlings were collected from Cos plants from two different cultivars. These were grown gnotobiotically to assess vertically transmitted microbial contributions and compared to commercial leaf lettuce. Culture independent next generation T-RFLP, 16S, and ITS illumina sequencing were performed along with traditional culture-dependent methods to determine genus identities and their relative abundance in each tissue type. Results to date suggest distinct microbiomes in seeds, seedlings, and leaves along with a genotype-dependent shared core microbiome. Abundance analysis in various plant compartments points to a putative pathway for dominant seed endophytes and suggests they have a temporal and functional role in the plant holobiont.

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INTERSPECIFIC PHOSPHORUS FACILITATION VIA RHIZOSPHERE PROCESSES IN HERBACEOUS SPECIES UNDER DEFOLIATION AND PHOSPHORUS ADDITION

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Interspecific facilitation is common under resource and non-resource stress, but how interacting species with different life histories (conservative vs. competitive strategy) response to phosphorus (P) deficiency and heavy defoliation is still unclear. To characterize belowground strategies related to P acquisition, we combined species that vary in their P-acquisition strategies at two P levels. We hypothesized that combinations of species that acquire P more efficiently are better adapted to P-impooverished soils and defoliation. Combinations that benefit from P amendment are expected to perform better on soils with a high P level. A pot experiment was conducted with five monoculture and ten mixture combinations, and 11 root functional traits related to P acquisition were selected. The root traits of *Stipa grandis*/*Leymus chinensis*, *S. grandis*/*Cleistogenes squarrosa*, *S. grandis*/*Artemisia frigida*, *L. chinensis*/*A. frigida* comprise combinations usually inefficient for P acquisition, i.e. low specific root length, root hair length, acid phosphatase activity or carboxylate concentration in the rhizosphere; their aboveground biomass is enhanced under P amendment. Neighbored with *Carex korshinskyi*,

a species with competitive strategies for P acquisition, and combinations of *L. chinensis*/*C. squarrosa* and *A. frigida*/*C. squarrosa* were better adapted to defoliation and P-deficient soil via finer roots and greater acid phosphatase activity or carboxylate release in the rhizosphere. The associations with *C. korshinskyi* usually had greater leaf manganese concentration ([Mn]) on P-deficient soils, and greater leaf [Mn] with *A. frigida* on P-enriched soils, especially under defoliation. This indicates these species facilitated P uptake of their neighbors by mobilization of sorbed P through the release of carboxylates in their rhizosphere. Our study shows the combinations of species that are efficient at P acquisition depends on soil P level; it highlights the importance of acknowledging interspecific P facilitation via rhizosphere process on both P-impooverished and P-enriched soils.

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BACTERIAL MICROBIOME IN WHEAT, CANOLA, AND LENTIL GENOTYPES

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Plants are holobionts, single dynamic entities in which microorganisms provide a large portion of the genetic information that influences plant function. These microorganisms in agricultural systems offer the potential of improving crop production and yield, as well as protection against abiotic and biotic factors such as climate change, pests, and pathogens. Wheat (*Triticum aestivum*), canola (*Brassica napus*) and lentil (*Lens culinaris*) are staple crops in Western Canada that significantly contribute to world food supply. New knowledge regarding the microbiome of these crops will give new perspectives and strategies for plant breeding in which breeders select for beneficial plant associated microbial communities. Seeds are the source of origin in most plants and are essential structures that determine next generation's traits. We characterized the seed microbiome from fifteen wheat, canola and lentil genotypes grown in the same location in 2016 in Saskatchewan, Canada. Seeds planted (original) and harvested (offspring) samples, were subjected to DNA extraction, high-throughput amplicon sequencing of the 16S rRNA gene, reads processing, and assembling into single nucleotide variants (SNVs). We found SNVs common among all genotypes suggesting there is a core bacterial community common among crops, mainly comprised of members of Enterobacteriaceae, Microbacteriaceae, Sphingomonadaceae, and Nocardiaceae families. Currently we are analyzing the preservation and inheritance of this bacterial community and the relationship with the root microbiome.

P.168**GRAZING INTENSITY MODIFY MICRO-FOOD WEBS VIA ALTERING PLANT ROOTS IN THE TIBETAN ALPINE MEADOW****B Wan**¹, X Mei¹, Z Hu¹, W Fan¹, H Guo¹, X Chen¹, F Hu¹, M Liu¹¹*Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu Province, China*

Grazing plays a critical role in influencing community structure and productivity in natural grasslands. To date, the effects of grazing on grassland aboveground plant community and belowground microbial community composition have mainly been studied, however, there is litter information on the effect of grazing density on grass root quantity and quality, and following effects on micro-food webs. Taking advantage of a 5-year field experiment that controlled grazing disturbance density via fence enclosures in the Tibetan plateau meadow, we explored the effects of grazing intensity on root traits, soil micro-food web and their linkages. Our results showed that the changes of grass root quantity and quality played the pivotal role in mediating soil food web through altering microbial and nematode communities under different grazing pressure. Moderate grazing disturbance increased significantly grass root biomass and root nitrogen concentration, mainly due to the compensatory growth of dominant plant species, while this stimulatory feedbacks could not be sustained at heavy grazing intensity. Also, moderate grazing increased root herbivorous numbers and fungal biomass but had no effects on bacterial biomass, which could regulate the growth of higher trophic groups, resulting in higher fungivorous numbers. Further, network analysis of nematode community showed that more number of edges and higher clustering coefficient were observed under moderate grazing disturbance, which implied moderate grazing strengthened the soil micro-food web community complexity and structure. The structure equation models showed that the lower trophic groups were directly influenced by bottom-up controls of root traits, while the higher trophic groups with larger body size were mainly affected by soil property. Together, our results suggested that the rational grazing intensity could be adopted as an effective management for recovering the degraded grasslands.

Keywords: Alpine meadow, grazing intensity, soil food web, rhizosphere, soil community, Tibetan plateau

P.169**ROOT BLOTTING METHOD FOR NON-DESTRUCTIVE SPATIAL ANALYSIS OF PHOSPHATASE ACTIVITY IN THE RHIZOSPHERE****V Lin**¹, J Rosnow^{1,3}, D Smercina^{1,2}, J Moran¹¹*Pacific Northwest National Laboratory, Richland, WA, United States*²*Michigan State University, East Lansing, MI, United States*³*Donald Danforth Plant Science Center, St. Louis, MO, United States*

Phosphorus (P) is an essential nutrient for plant growth, although much of the phosphorus in soils may not be bioavailable depending on pH and geochemical environment. Understanding how plants access P from their environment is essential to evaluating the growth potential and productivity of plants, particularly in nutrient poor systems. Organic P sources, such as P contained within organic matter, can act as a major source of P in

some soil systems. Phosphatases, which act on organic P to liberate inorganic phosphate, are produced by both plants and microbes and are considered one of the most active classes of enzymes in soil. We have developed a root blotting method to image phosphatase activity in the rhizosphere in a spatial manner. Proteins from the rhizosphere can be transferred to a nitrocellulose membrane with retention of enzymatic activity and spatial distribution. Subsequent application of a fluorogenic phosphatase indicator, DDAO phosphate, enables visualization of areas with high phosphatase activity. The proteins can then be fixed to the membrane, and treatment with a fluorescent total protein stain (SYPRO Ruby blot stain) allows for visualization of total protein distribution. Taken together, the images of root phosphatase activity and total protein localization can be mapped back to the root architecture and provide insight into factors affecting the spatial distribution of enzymatic activity and protein accumulation in the rhizosphere. Notably, this method can be applied to plants growing in rhizoboxes containing sand or soil and can be performed multiple times in a non-destructive manner. We anticipate that this fluorescent indicator imaging technique on root blots can be used in diverse plant-microbe-soil systems to better understand the role of phosphatases in P acquisition and soil P cycling.

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EVALUATION OF COLONIZING ABILITY OF FIVE TEMPERATE RAINFOREST PROTEACEAE SPECIES GROWING IN YOUNG VOLCANIC SUBSTRATE

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Southern South American Proteaceae thrive in young volcanic substrates, which are extremely low in plant-available phosphorus (P). Most species belonging to Proteaceae exhibit an important nutrient-acquisition strategy based on the release of carboxylates through specialized roots named cluster roots (CR). Some Proteaceae have a recognized colonizing ability, which has been related to CR functioning. However, physiological functioning of other Proteaceae to thrive on recent volcanic substrates is unknown. We conducted an experiment where seedlings of five Proteaceae (*Gevuina avellana*, *Embothrium coccineum*, *Lomatia hirsuta*, *L. ferruginea* and *L. dentata*) were grown in three volcanic substrates. Two of the substrates are very poor in nutrients and were collected from the last deposits of the volcanoes Choshuenco and Ensenada. The other substrate also corresponds to a soil originated from volcanic materials, but exhibits high nutrient availability (named Santa Rosa, used as a control). We evaluated morphological responses (*i.e.*

height, dry biomass and CR formation), seedling performance (maximum quantum yield of photosystem II (F_v/F_m)), leaf nitrogen and P concentrations and carboxylates exuded by roots. The results showed that, in general, in Choshuenco and Ensenada substrates *G. avellana* and *E. coccineum* presented the highest height, biomass and leaf P concentration. Besides, *E. coccineum* was the only species that did not diminish F_v/F_m in the nutrient-poor substrates. *Gevuina avellana* was the species that presented the highest CR biomass and carboxylate-exudation rate (mainly succinate), whereas *Lomatia* species allocate less biomass to CR formation and exuded only oxalate. *Embothrium coccineum* had more CR biomass than *Lomatias*, but exuded carboxylates at a slower rate than these species, probably because at the time of the plant harvest (autumn), most CR had senesced in this species. We conclude that Proteaceae differ in their functioning and that *G. avellana* and *E. coccineum* are better colonizers than *Lomatia* species in recent volcanic substrates.

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USING POSITRON EMISSION TOMOGRAPHY TO STUDY MECHANISMS OF BACTERIA-MEDIATED PLANT GROWTH PROMOTION IN RICE

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Studying plant-microbe interactions (PMI) is essential to understanding mechanisms of plant growth promoting bacteria, particularly those associated with major crops. *Rhizobia*-legume interactions are relatively well characterized, however, other beneficial PMI are not as well understood. The aim of this work is to determine the mode of action of *Herbaspirillum huttiense* L4.2 when associated with rice. This will be accomplished by: i) utilizing an imaging system that allows for visualization and quantification of radioactive tracers and ii) analyzing confocal images of green fluorescent protein (GFP)-tagged cells.

Using radioisotopes and positron emission tomography (PET) detectors designed specifically for plants (PhytoPET) is a novel approach to studying PMI. This technique provides information on biological processes and facilitates real-time visualization of radioactive tracers throughout plants. This enables visual comparison and quantification of differences in resource uptake and allocation between inoculated and control plants. To study the translocation of glucose in rice we used the structural analog 2-deoxy-2-[¹⁸F] fluoroglucose (¹⁸FDG). Preliminary results showed that ¹⁸FDG was incorporated into leaf tissue, as the radiotracer could be visualized moving through the shoot; however, thus far, it could not be detected in the roots. A promoterless GFP was inserted into *H. huttiense* L4.2 via transposition, consequently GFP expression among transconjugates was dependent on insertion site. Confocal microscopy revealed that one isolate showed greater fluorescence on the plant roots, though, all showed similar patterns of colonization. In each case, *H. huttiense* L4.2 was most abundant at the junction between primary and secondary roots and was occasionally observed in between plant cells and/or along the length of root tissue. These experiments were necessary to provide a foundation for further investigation into the plant growth promoting effects of *H. huttiense* L4.2.

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POEM: A GRASSLAND FIELD EXPERIMENT TO SHED LIGHT ON THE BELOWGROUND MECHANISMS OF PRIORITY EFFECTS

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Alongside plant species and functional group diversity, both the order and timing of arrival of plant species during plant community assembly can have long lasting impacts on plant community structure and functioning. This phenomenon is referred to as a priority effect and is related to the idea of historical contingency. Priority effects occur when early arriving species affect the establishment, growth, or reproduction of species arriving later. Although such priority effects frequently occur during assembly of natural plant communities, we still know very little about the mechanisms responsible for these effects (e.g., resource preemption, plant-soil feedbacks, species complementarity, role of the plant-associated microbiome, etc.). POEM (PriOrity Effect Mechanisms) is a research project aiming to fill this gap. Using a grassland field experiment that manipulates plant functional group order of arrival, we are exploring the relative roles of state-of-the-art theories of species coexistence in the creation and persistence of priority effects in dry acidic grasslands. Depending on the functional group that arrives first at a site (either grasses, forbs, or legumes), the early-arriving species will have potentially different effects on the soil environment (water and nutrient uptake, soil and plant-associated microbiota, root exudation, and nitrogen facilitation), such that later-arriving species will not experience the same conditions. Therefore, we hypothesize that these differences in plant functional group order of arrival will lead to communities differing in structure and functioning aboveground and belowground. To investigate this hypothesis, we are looking at how (1) root niche partitioning between grassland species, (2) root productivity, root turnover, and vertical root distribution at the community level, (3) plant-soil feedbacks, and (4) soil and plant-associated microbiomes are affected by priority effects during plant community assembly. Such new knowledge has also much potential for future application in restoration and management of dry acidic grasslands.

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BIOCHAR MEDIATED EFFECT ON ROOT DEVELOPMENT IN MAIZE COWPEA INTERCROP

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Mechanisms of biochar mediated increase in crop yield still seem not fully understood. Biochar effect on root morphology and architecture could lead to unraveling the possible effect of biochar on plant growth enhancement. Since root development is very fundamental in nutrient and water uptake and overall

functioning of the plant and more so in engineering the associated microbial community that could enhance plant growth, evaluating effect of biochar on roots is very essential. This research evaluated the effects of three rates of biochar with or without cattle dung or NPK fertilizer under four cropping patterns on maize and cowpea root development in a field experiment in North-West University, Mafikeng campus, South Africa. Pine wood Biochar was applied at 0, 2.5 and 5 t/ha three weeks after planting by making some depressions within the interrow spacing and covering it with soil. Other amendments were also applied the same time. Roots were harvested monthly from the date of amendment application and during harvest by destructive sampling. Using shovelometric score board, the root parameters were determined. Biochar amended soil had significantly higher root mass over the control. The root angle of both the first roots and crown roots were between 40 °C to 89 °C. The root length and number of crown roots were also significantly higher ($P < 0.05$) in biochar amended soil than the control. All these properties were enhanced by application of either cattle dung or NPK fertilization.

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SMOOTH BROME INVASION EFFECTS SOIL STRUCTURE AND ECOSYSTEM SERVICES

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Invasive species have severe impacts on ecosystems, including drastically altering the plant community which in turn impacts soil microbial communities. These changes can then have cascading effects on ecosystem services. Despite increasing knowledge of how invasive plants can change plant community structure and soil properties, little is known about how multiple invasive plants influence ecosystem services. To investigate this, 20-26 soil cores were collected weekly for 26 weeks in a grassland, near Saskatoon, Canada, undergoing invasion by smooth brome (*Bromus inermis*) and five other invasive species. Greenhouse gas emissions, nutrient cycling and forage biomass production were measured, as well as glyphosate degradation rates. Additionally, plant surveys were conducted weekly in each in each plot.

Smooth brome increases aggregate size and decreases soil moisture. Forage biomass, GHG emissions, nutrient cycling and glyphosate degradation varied seasonally and was primarily driven by changes in aggregate size and moisture. Smooth brome invasion has impacts on soil structure which in turn, drive changes ecosystem services. Changes in soil structure will have lasting impacts on this ecosystem and make restoration of native grassland difficult, leading to long-term changes in ecosystem functioning.

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RESPONSE OF RAINFED MAIZE ROOT MORPHOLOGY TO PLANT POPULATION UNDER NO-TILLAGE

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Plant morphology is used as an indicator to assess growth response to agronomic practices such as plant population and soil tillage. Although aboveground maize growth response to agronomic practices is well-

researched, a paucity of data exists on how interplant competition influences maize root morphology. The aim was to quantify the effects of plant population on rainfed maize root morphology under no-tillage.

A trial was established on sandy loam soil during the 2017/2018 growing season near Ottosdal, North-West Province, South Africa. Treatments were three plant populations (20 000, 30 000 and 40 000 plants ha⁻¹) established at 0.76 m row spacing. Experimental design was randomised block design with three replicates. Acrylic minirhizotron tubes were installed after planting at a 45° angle, centred, and parallel with two adjacent rows. Digital images were taken at 15 cm increments to 60 cm soil depth, using a CI-600 *In Situ* Root Imager minirhizotron at the sixth leaf collar, tasselling and full flowering growth stages. Digital images were analysed using *RootSnap!* software.

At 0-15 cm depth, 30 000 plants ha⁻¹ had a higher ($p < 0.05$) total root count than 40 000 plants ha⁻¹ at tasselling and full flowering. Interestingly, total root length remained constant ($p > 0.05$) between treatments at this depth. Embryonic and postembryonic shoot-borne roots accounted for 81% of the total root length at 30 000 plants ha⁻¹, while only 64% at 20 000 and 40 000 plants ha⁻¹. At full flowering, 40 000 plants ha⁻¹ had higher ($p < 0.05$) total root volume at 0-15 cm depth compared to 30 000 plants ha⁻¹, while no significant differences were found at deeper depths. This indicates shallow root morphology was more strongly influenced by plant population compared to deeper located roots.

Interplant competition influence maize root morphology differently at distinct soil depths, highlighting the importance of nutrient management under no-tillage.

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INTEGRATING PLANTS, ROOTS, AND SOIL NEMATODES TO RESTORE NORTHERN PRAIRIE ECOSYSTEMS

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Changes in climate and the intensification of human land uses threaten grasslands around the world. Until recently, evaluating grassland restoration focused on the structure, diversity, and composition of plant communities above-ground. However, biotic interactions below-ground are emerging as key drivers of the function of restored grasslands. Our objective was to relate changes in the architecture and morphology of roots with the structure of soil food webs in revegetated mixed-grass prairies. We selected two dominant plant species, *Pascopyrum smithii* (western wheatgrass) and *Bouteloua gracilis* (blue grama), and measured their roots and associated soil food webs along a chronosequence of revegetated fields in Grasslands National Park, SK (Canada). For each species, we compared the architecture and morphology of its roots with the diversity and structure of soil nematode communities. Roots in recently revegetated fields had increased length, surface area, volume, and number of tips, and were associated with a high proportion of root-feeding nematodes. In contrast, bacterial feeding nematodes were more abundant in the rhizosphere of plants in undisturbed prairies, characterized by shorter, less branched roots with reduced total volume and surface area. While total root biomass and levels of soil carbon in revegetated fields started to resemble those from the undisturbed prairie, the diversity and structure of soil nematode communities was not restored 13 years after revegetation. Our results indicate that root traits can help explain structural and functional changes in plant and soil communities

over the course of restoration and can be used to refine measurements of restoration success in grassland ecosystems.

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DEVELOPMENT AND UTILIZATION OF 2-DIMENSIONAL HIGH-THROUGHPUT PHENOTYPING PLATFORM FOR GENETIC ANALYSIS OF ROOT SYSTEM ARCHITECTURE

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In the modern era of quantitative genetic research, great progress has been made in genotyping platforms in terms of economical availability and applicability. Precise and high-throughput phenotyping still remains a challenge for crop plants and specifically for root topology and architecture traits. Here we report on the development and utilization of a two-dimensional high-throughput platform to phenotype root traits in a pouch-based hydroponic growth system. Along with quantifying root growth and topology traits, this allows for the study of root system architecture (RSA) traits such as root system depth, width and convex area simultaneously. This platform also provides the opportunity to phenotype root traits in a dynamic fashion as the plant grows and develops. The throughput for a single system is 90-100 plants per day. The rationale behind development of this system is to acquire high quality root images with better contrast, which facilitates capturing images of the finer roots in the root system, allowing more accurate and precise conversion of digital to quantitative root trait data. This system has been validated for its efficiency to capture significant root architectural differences among diverse genotypes of wheat and soybean. This sets the stage for deeper studies of genetic variation and joint Linkage-Genome Wide Association Studies in wheat and soybean to map genomic regions that are associated with root traits of interest. Eventually results from these types of studies should provide the linked markers and possibly gene-specific markers that can be used by breeders to manipulate root architectural traits to achieve higher yielding and more stress tolerant crops.

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MYCORRHIZAL FUNGI ABUNDANCE AND COMPOSITION ASSOCIATED WITH PLANT ROOTS ACROSS THE ATLANTIC REGION

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Mycorrhizal fungi abundance and composition associated with plant roots across the Atlantic region. Soil sampling will be conducted in primarily in agricultural production in the four Atlantic Provinces. The ~500 sampling points will be selected with the conditioned Latin hypercube method. Roots will be collected from fresh soil samples taken at 0-15 cm depth for all sampling locations across the Atlantic region. Roots will be

washed clean of adhering soil and processed for mycorrhizal characterization. Mycorrhizal associations with roots will be assessed using both microscopy and next generation sequencing. For microscopy quantification of endophytic mycorrhizal colonization roots (such as arbuscular and ericoid mycorrhizal fungi), fine roots will be randomly sampled and cleared and stained for microscopy quantification of the percent root length colonization. Root tips of woody species will be collected fresh to quantify the percent colonization of root tips under a dissecting scope and score the variation in morphological types using a grid intersect quantification and 'morphotyping' descriptive methods. Root fragments not processed for microscopy will be used for molecular characterization of rhizosphere fungal communities using the general fungal primers to amplify the fungal ITS2 region for Illumina MiSeq sequencing. The relative abundances of taxonomic groups of fungi found associated with roots will be used to assess the diversity of mycorrhizal fungi associated with plant roots.

The next generation sequencing data in combination with more formal microscopy will provide a comprehensive assessment of the mycorrhizal diversity across different ecosystems and land use types in Atlantic Canada, and will allow examination of the linkages between local climatic, soil and anthropogenic landscape factors to the composition and function of mycorrhizal communities. Outcomes: Assessment of the mycorrhizal fungi diversity using microscopy and next generation sequencing data across Atlantic Canada.

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"RHIZO-GEL 3D" A SYSTEM TO STUDY THE THREE-DIMENSIONAL ROOT SYSTEM ARCHITECTURE OF ARABIDOPSIS

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The simplicity of the Arabidopsis root system combined with its easy cultivation in the laboratory became it as the model organism to study plant roots around the world. The extraordinary morphological flexibility of its root system architecture to change in response to nutrient availability in the media, together with its enormous genetic diversity and the increasing list of molecular tools designed for this plant, place Arabidopsis as the most used living system to study molecular mechanisms that improve the root system architecture performance of crops. An example is the current breeding programs that are employing Arabidopsis to understand the molecular mechanisms of genes that control the root longitudinal-growth and the lateral root formation and their growth angle to increase nutrient acquisition and drought tolerance of crops. However, the current scientific reports show that the root architecture system analysis of Arabidopsis is based exclusively two-dimensional analysis, leaving aside the adaptive meaning of the three-dimensional structure as well its potential use to improve the root system architecture in crops. For all above, following the Root-Soil-Microbiome Interactions (RSMI-GIFS) research team propose and taking advantage of its resources and extensive experience interpreting the three-dimensional structure of crop roots, we decided to create "Rhizo-gel 3D". Rhizo-gel 3D is conceived to perform high throughput studies of the three-dimensional root system architecture of Arabidopsis through its different plant life stages. During the Rhizosphere 5 meeting, I will describe the Rhizo-

gel 3D components of our prototype, as well, the results of its use and its potential application to study the rhizosphere interactions that determine the root system architecture in Arabidopsis.

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NEIGHBOUR DIVERSITY AFFECTS THE RHIZOSPHERE MICROBIOME ASSEMBLAGES OF TREE SPECIES PAIRS IN SUBTROPICAL FOREST ECOSYSTEM

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Introduction: Soil microorganisms are essential to plants for the cycling and uptake of nutrients. Previous research has shown that subtropical forests harbor a great amount of microbial diversity. However, the role of neighbourhood diversity and the plant mycorrhizal trait on the rhizosphere microbial diversity are not well understood. Thus, this project studied the rhizosphere soil microbial composition of tree species pairs in a Chinese subtropical forest ecosystem.

Methods: Rhizosphere soil samples were collected using soil cores, freeze-dried and microbial genomic DNA was extracted. The bacterial (V4 region of 16S rRNA) and fungal (ITS2 fragment of rRNA) amplicon libraries were sequenced using a paired-end approach with Illumina MiSeq. Bioinformatic analysis of the sequence data was performed using mothur based custom pipeline. The specific research questions were tested by R based statistical analysis.

Results: PERMANOVA analysis revealed a significant effect of tree neighbourhood diversity on both fungal and bacterial communities ($P < 0.003$ & $P < 0.008$ respectively). Tree mycorrhizal type has shown significant effect on fungal communities ($P < 0.001$) whereas there was no significant effect on bacterial communities.

Conclusions: Neighbourhood diversity affects the rhizosphere microbiome as compared to tree mycorrhizal type in this particular ecosystem.

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EFFECTS OF BIO-ORGANIC AND CHEMICAL FERTILIZER ON THE ROOT SYSTEM ARCHITECTURE OF PEAR (PYRUS PYRIFOLIA)

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Root system architecture (RSA) is a central determinant of root function and thus plant productivity. RSA is highly plastic, responding to nutrient availability and the heterogeneity of the soil environment. In order to study the effects of a localized supply of bio-organic and chemical fertilizer on the root system architecture of pear trees, a split-root experiment was conducted with 2-year old 'Cuiguan' (*Pyrus pyrifolia* Nakai). No fertilizer (NF), chemical fertilizer (CF), and bio-organic fertilizer (BIO) were used in the following combinations: NF-NF, NF-CF, NF-BIO, CF-CF, BIO-BIO, and BIO-CF. Except for the NF treatment, roots in each chamber were supplied

equal amounts of N, P, and K. Shoot growth was measured at 210 days after budding and the 2nd lateral roots, which were approximately 20-cm in length, were randomly collected from each chamber, photographed, and analyzed for RSA parameters by SmartRoot. Results showed that in BIO containing chambers (NF-BIO, BIO-BIO and BIO-CF), the lateral root number, density, activity, and total lateral root length (TLRL), total lateral root surface area (TLRS), and total lateral root volume (TLRV), were significantly higher than in non-BIO containing chambers. Root growth was significantly higher in NF-BIO chambers than in NF-NF. In the BIO-CF chambers, TLRL, TLRS, and TLRV were significantly increased over those in the CF-CF chambers; in addition, the BIO-CF mix resulted in trees with greater trunk girth, without a reduction in height, over trees grown in CF-CF or BIO-BIO. In conclusion, application of bio-organic fertilizer resulted in a denser RSA than application of CF alone or NF, and the combination of BIO and CF synergistically enhanced RSA, which in turn resulted in increased trunk girth without the sacrifice of tree height.

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CADMIUM CONCENTRATION IN BARLEY RHIZOSPHERE IN A CD CONTAMINATED SOIL AND THE ROLE OF PGPR IN PLANT TOLERANCE TO CONTAMINATION

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Cadmium concentration in barley rhizosphere in a Cd contaminated soil and the role of PGPR in plant tolerance to contamination

Today, phytoremediation is used to reclamation of heavy metals contaminated soils and it is an economical agricultural operation. The inoculation of PGPRs improves plant growth in contaminated soils and increases the phytoremediation efficiency. This experiment was carried out in Rhizoboxes to evaluate the effect of PGPR inoculation on barley growth in Cd contaminated soil. Irrigation water salinity (control and 8 dS /m), PGPRs (control and *Pseudomonas*), cadmium contamination (4 mg Cd/ kg and 31mg Cd/ kg) were considered as treatments. Six Barley seeds were cultivated in Rhizoboxes designed by Wenzel (2009). After plant growth and forming a dense root in the lower part of rhizoboxes, the rhizosphere soil was cut using a suitable instrument at intervals of 1 mm. Then, some indices were measured in soil and plant. The results indicated that increasing cadmium, decreased shoot and root dry weight, while shoot Cd content increased. Cadmium concentrations in inoculated plants were lower compared to non-inoculated ones. Also the lowest concentration of cadmium was recorded in Co- inoculation of *Pseudomonas* and *Glomus versimorum* treatment. PGPRs supported barley plant in Cd contaminated soil and increased phytoremediation efficiency

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EFFECTS OF PHOTOSELECTIVE NETTING ON ROOT GROWTH AND DEVELOPMENT OF YOUNG GRAFTED ORANGE TREES UNDER SEMI-ARID CLIMATE

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Photoselective netting is well-known for filtering the intercepted solar radiation affecting light quality. While its effects on above-ground of plants have been well investigated, the root system was neglected. Here, we evaluated the effects of photoselective netting on root growth and plant development. Minirhizotron and ingrowth cores were applied in a field experiment, performed in a 4-year-old orange orchard grown under three different photoselective net treatments (red, pearl, yellow) and an unnetted control treatment. Our observations confirmed the significant positive effect of photoselective nets on photosynthesis rate, vegetative growth and fruit yield. Trees grown in the pearl plot developed evenly distributed root system along the observation tubes while trees in control, red and yellow plots had a major part of roots concentrated at different depth ranges of 60-80, 100-120, and 120-140 cm, respectively. Photoselective nets showed a strong impact on shoot-root interaction and proved equally successful in promoting rapid establishment and early high-fruit yield in young citrus trees. However, at long-term effect, yellow net might outperform because it could enable plants to develop deeper root systems, which will uptake water and nutrients more efficiently in semi-arid areas with sandy soil.

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ARSENIC SPECIATION AND TRANSPORT IN THE RHIZOSPHERE OF AN ARSENIC-HYPERACCUMULATING FERN

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Anthropogenic enrichment of toxic metal(loid)s, including arsenic (As), in soils poses hazards to humans and ecosystems globally. The arsenic-hyperaccumulating fern *Pteris vittata* accumulates high concentrations of arsenic in its fronds, and can be used to decontaminate soil with minimal site disturbance. However, current remediation times are prohibitively slow. To increase remediation rates, rhizosphere processes mobilizing arsenic for uptake into the fern must be better understood, and possibly optimized through soil treatments that increase arsenic phytoavailability. However, those could alternately lead to arsenic leaching.

The objective of this study was to determine the effects of (i) phosphorus (P) and (ii) mycorrhizal fungi inoculant addition to soil on arsenic uptake and leaching in the *P. vittata* soil-plant system. In a soil column study, *P. vittata* was planted in a sandy loam soil historically contaminated with arsenic (114 mg/kg). Soil was treated either with phosphorus or mycorrhizal fungi inoculant. Over 22 weeks, synthetic rain was eluted through columns. Arsenic, iron (Fe), phosphorus, and dissolved organic carbon were tracked during growth in soil

porewater, column effluent, and plant tissue. Arsenic and iron speciation was determined in soil and root samples using bulk and micro-focused synchrotron X-ray absorption near edge spectroscopy (XANES).

Fern arsenic uptake outpaced loss to leaching, and was attributed mainly to arsenic mobilization directly at the root-soil interface, not porewater transport of arsenic. Arsenic leached from control and treated columns, with the highest concentrations and total amount lost from phosphorus-treated soil. Phosphorus treatment decreased total arsenic accumulated in the fern. Speciation by XANES indicated less than 30% arsenic (III) and iron (II) species in rhizoplane soil, but up to 80% arsenic (III) in roots. We conclude phytoextraction can cause arsenic leaching from bulk soil but is limited to rhizosphere soil, with arsenic mobilized primarily *via* ion exchange and subsequently reduced within the root.

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CARBON DISTRIBUTION OF RHIZOSPHERE THROUGH PLANT

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Carbon-based compounds, such as monosaccharide and acids which fed the microorganisms, are transported through phloem to the rhizosphere. However, the precise destination and use (growth, maintenance or exudation) of these carbohydrates are difficult to predict, as they depend on the plant architecture and development over time. Functional-structural plant models (FSPMs) can help trace the carbon movements in the whole plant, as they are able to simulate 4D plant-soil interaction with high temporal and spatial resolution. Last but not least, such tools can be used to compare simulations with experiments, to better understand plant-rhizosphere interactions.

Here, we would like to present a new FSPM, CPlantBox that could be used to perform such simulations. CPlantBox, an extension of the CRootBox model, is a model that can simulate the 3D growth and development of full plants (roots and shoot). To be able to model carbon flow in the whole plant, CPlantBox was coupled to a model of phloem and xylem flow based on Münch theory (PiafMunch). By doing so, we are now able to simulate the flow of carbohydrates in complex plant structures and therefore theoretically predict the distribution of root exudates. Currently, we have validated the CPlantBox-PiafMunch coupling by comparing the simulated carbon flow with experimental data. Now, we are planning to use the models to estimate root exudation locations and volume

In most cases, rhizosphere scenarios are difficult to reproduce, measure and analyze because they are root-dependent and time-varying. Applications of fast and efficient plant simulations have the potential to supplement rhizosphere experiments and help interpret them. We are looking forward to using CPlantBox to understand rhizosphere with better temporal and spatial resolution.

P.186**THE GREENER PROJECT: RHIZOREMEDIATION COUPLED WITH MULTI-SYSTEM APPROACHES FOR BIOREMEDIATION.****K Germaine**¹, D Dowling¹, X Germaine²¹*Institute of Technology Carlow, Carlow, Ireland*²*MicroGen Biotech Ltd, Carlow, Ireland*

The GREENER project (an acronym that is short for InteGRated systems for Effective ENvironmEntal Remediation) involves 17 European and four Chinese partners and has a total budget of just under €7 million. It is part of a flagship EU-China collaboration program designed to stimulate research co-operation between Europe and China. Both Europe and China have serious environmental pollution issues. For example, just on the island of Ireland, it is estimated that there are about 2000 sites considered to be polluted by the Environmental Protection Agency (EPA). Traditional methods of treatment such as 'dig and dump' are expensive, destructive to local ecosystems and so not very practical for treating large polluted sites. The GREENER project will investigate the use of bioremediation (using plants, bacteria and fungi) to remove toxic pollutant from soil, air and water. Bioremediation has been around for decades, and has the advantages of being relatively cheap and environmentally friendly. However, its use in treating environmental pollution is limited due to the fact that it is a slow process and the range of pollutants that it can be used to treat is quite limited. The GREENER project will look at the use of synergistic bioremediation technologies in conjunction with other physical treatment methods (e.g. electrochemistry) to effectively remove pollutants from water and soil/sediments, while generating side products of interest, such as bioelectricity. Fundamental research will be performed at lab-scale, while pilot-tests will be used to prove the feasibility and cost effectiveness for large scale clean-up applications both in Europe and in China.

P.187**A HYBRID PARTIAL DIFFERENTIAL EQUATIONS – CELLULAR AUTOMATON MODEL FOR EMERGENT STRUCTURES BRIDGING SCALES****A Prechtel**¹, R Schulz¹, A Lieu¹¹*Department of Mathematics, Erlangen, Germany*

We present a novel, comprehensive model on the pore scale to describe the dynamic self-organization of soil aggregates in the rhizosphere influenced by various attracting forces, geochemistry, and microbiology. This mechanistic model can then be upscaled to the root system scale.

On the pore scale a discrete cellular automaton (CAM) is combined with continuous partial differential equations (PDEs) for structure evolution (distribution of phases including mucilage, air, root hairs and aggregates) and reactive species and charges in solution. Then (formally and operationally) periodic homogenization allows to infer soil functions / parameters for evolving rhizosphere microstructures: e.g., effective diffusivity, or permeability. Note that the basis for upscaling is not image analysis but a mechanistic, process-based dynamic model. This information on effective functions from the microscale at potentially every grid node can then be used on the macroscopic level in continuous reactive multicomponent transport and flow

equations (fully coupled PDE systems). Of particular interest in the rhizosphere are properties of mucilage as a separate, dynamic, viscous phase, or root hairs as lower dimensional objects affecting the structure of solid particles.

We use efficient and accurate numerical methods to treat the fully coupled micro-macro problems appropriately in a massively parallel environment: local discontinuous Galerkin discretization (flexible, handle discount. due to evolving geometry, few DOF, local), and implicit/explicit solution algorithms, inexact Newton methods; in an object oriented, scalable C++ code. Simulations demonstrate the aggregate formation for prototypic building units, and the derivation of structural information as average particle sizes or compactness.

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TRANSFERABILITY OF RADIOCESIUM CONTAINED IN BURIED ROOT MAT CONTAMINATED BY FUKUSHIMA DAI-ICHI NUCLEAR POWER PLANT ACCIDENT

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To reduce radiocesium transfer from soil to pasture plant, inversion tillage to replace contaminated top soil with subsoil was conducted in pastures contaminated by the Fukushima Dai-ichi Nuclear Power Plant accident. However, the long-term migration and retention dynamics of radiocesium contained in the root mat layer buried in the lower soil has not been well known. To clarify the dynamics, we collected samples of surface soil, buried root mat layer (BRM layer), and soil below the layer from three pastures, where inversion tillage was conducted from 2012 to 2013, on August 2017. Radiocesium concentration and soil characteristics including biochemical ones were analyzed for the samples. The transferability of radiocesium from the collected samples to grass (*Dactylis glomerata* L.) was also evaluated by pot cultivation experiment. The BRM layer was found in depths of 20 to 30 cm at all sites and contained radiocesium in more than ten times higher than the surface and the below soils. Compared to the surface soil, the BRM layer had higher total carbon content and lower pH. Diversity index of bacterial communities and organic matter decomposition activity of microbial communities were also lower in the BRM layer than in the surface soil. Those results suggest that organic matter decomposition activity of the BRM layer was reduced due to loss of the diversity of the microbial community by the changes in physical and chemical properties after inversion tillage. The radiocesium in BRM layer also had higher transferability to grass in comparison to that in the surface soil. From those results, it was concluded that the BRM layer has stably retained radiocesium, which has high transferability to plant, for a long time.

This work was carried out under a contract with the Aomori Prefectural Government, Japan.

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ROOT EXUDATION OF COUMARINS FROM SOIL-GROWN ARABIDOPSIS THALIANA IN RESPONSE TO IRON DEFICIENCY

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Iron (Fe) is an essential nutrient for plants. However, in calcareous soils, the bioavailability of Fe is limited. Plants developed at least two strategies to overcome this limitation: a chelation-based mechanism which involves exudation and uptake of phytosiderophores (strategy II) and a reduction-based mechanism (strategy I) which involves acidification of the rhizosphere, reduction of Fe(III) and uptake of Fe(II). Recent studies have highlighted the importance of coumarins in Fe acquisition by strategy I plants (in particular *Arabidopsis thaliana*) in hydroponic solutions or on artificial substrates. This study aimed to characterize the exudation of coumarins from plants grown on Fe limited soils.

Six soils varying in carbonate content, pH and DTPA-extractable Fe concentrations were selected for two pot experiments. Plant-induced chemical changes in the rhizosphere were assessed in the soil solution throughout the duration of the experiment (experiment 1) and root exudates were sampled hydroponically after carefully washing the roots (experiment 2). Scopoletin was the predominant coumarin found in the exudates of *A. thaliana* grown on the different soils. Exudation rates of scopoletin tended to increase with decreasing bioavailability of Fe in soil, with the exception of the acidic soil where plants showed exudation rates similar to the rates measured on alkaline soils. In a follow-up experiment, another sampling approach has been implemented, using a rhizobox in combination with a root exudate collecting tool. This set-up allowed less invasive and repeated sampling of exudates from an undisturbed root mat. Results from this experiment are currently under evaluation.

This is the first study reporting exudation of coumarins from *A. thaliana* grown on natural soils with varying Fe availability. Root exudation sampling from soil-grown plants remains challenging and this study contributes to a better understanding of exudation patterns influenced by limiting Fe bioavailability in soils.

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WILLOW ROOT AND BIOMASS GROWTH IMPROVEMENT BY THE LIMING OF HEAVILY CONTAMINATED SOIL

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Improvement of willow (*Salix* spp.) growth is the most important factor to keep toxic elements phytoremediation effective on heavily contaminated soils. The application of soil amendments is usually required. Liming can be a suitable measure to decrease the element plant availability, resulted in sufficient plant development. Better plant growth is usually connected with the development of large root area and maximum soil penetration helps with the improvement of phytoremediation. The effect of liming on root development, biomass allocation and toxic element distribution in non-harvestable (coarse roots, fine roots, stumps) and harvestable plant parts (twigs and leaves) of *Salix × smithiana* was assessed at the end of a 4-year

pot experiment at heavily Cd, Pb, Zn, and As contaminated weakly acidic soil. Lime application by quick lime as well dolomite significantly decreased the concentrations of mobile Cd and Zn in soil, substantially improved biomass production in the third and fourth years of experiment, and root parameters. The element content in leaves, twigs, and coarse roots was slightly decreased at treated soil, stronger effect was found after the application of quick lime than dolomite, the application rate did not play important role in the element accumulation. Fine roots accumulated the highest contents of toxic elements. Their translocation to above-ground biomass increased as follows: $Pb < As < Zn \sim Cd$. In contrast to Cd and Zn, great differences in As and Pb were recorded after measurements of individual below-ground biomass (stumps < coarse roots < fine roots). The dose and source of liming had crucial effects on root anatomy. Lower rates allowed to produce the longest fine as well coarse roots with the largest surface areas. Growing willows in limed trace element-polluted soils is a suitable measure for the long-term stabilization of heavily contaminated soils.

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HYDROGEN PEROXIDE INVOLVED IN ROOT DEVELOPMENT UNDER ZINC DEFICIENCY STRESS IN MAIZE (ZEA MAYS.L)

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Zinc (Zn) is one of the essential micronutrients for plant growth and development. The objective of this study is to investigate whether hydrogen peroxide (H_2O_2) involve in plant root development under Zn deficiency. The maize variety ZD958 was selected to grow with nutrient solution culture. Roots and shoots were sampled at 10 and 15 days of transplant after treatments of 0 and 1 μ M supply. Zn deficiency reduced crown root growth. Accumulation of H_2O_2 in the meristematic and elongation zone of crown roots was found under Zn deficiency by chemical staining and fluorescent probe detection. The exogenous addition of H_2O_2 scavenger DMTU reduce the inhibition of crown root by Zn deficiency. In contrast, exogenous addition of H_2O_2 and catalase inhibitor increased the accumulation of H_2O_2 in roots and inhibited crown root growth. Transcriptomics and proteomics analysis showed that some key genes and proteins related to antioxidant enzymes and cell elongation were down-regulated in Zn deficient roots. It is suggested that hydrogen peroxide might involve in the inhibition of crown root growth in maize subjected to Zn deficiency stress.

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WILLOW ABILITY TO REMOVE POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) FROM SOIL AMENDED BY BIOMASS FLY ASH

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The PAHs are persistent organic contaminants consisting two or more benzene rings with toxic, mutagenic, teratogenic and carcinogenic properties. The PAH compounds are formed during the incomplete combustion. The biomass combustion in power plants can lead to the PAH accumulation in biomass ashes. The PAH-polluted biomass ashes could be a possible source of agricultural soil contamination. Phytoremediation could be an appropriate bioremediation approach to remove the PAHs from soils. A three-year pot experiment was conducted to compare the PAH removal from soil amended with biomass PAH-contaminated fly ash at non-planted (natural attenuation) and planted (phytoremediation by willow - *Salix × smithiana*) treatments. The PAH removal from ash-treated soil was compared with PAH-spiked soil. Analysis of PAHs was performed by gas chromatography coupled with a mass spectrometric detector (Agilent GC6890/MS5975) after a sample ultra-sonication and extract purification. Results showed that ash PAHs were similarly susceptible to the removal as spiked ones. Soil phytoremediation by willow was more efficient approach for the removal of low and medium molecular weight PAHs from ash-amended soil (70.5 and 63.6, respectively) than the natural attenuation. Total PAH removal from ash-amended soil (50.9%) and PAH-spiked soil (41.7%) planted by willow were significantly higher ($p < 0.05$) than from the non-planted soil but were not statistically different between each other. Since the second growing season the tested PAH level of soil contamination had no adverse effect on willow yield. PAH removal by willows was negligible within the range 0.01-0.16%. This could indicate that the willow used in our phytoremediation approach significantly boosted the PAH microbial degradation in rhizosphere.

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IONOMIC VARIATION IN PLANTS GROWN UNDER NUTRIENT DEFICIENCY WITH EMPHASIS ON MOLYBDENUM ACCUMULATION

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The ionome is defined as the mineral nutrient and trace element composition in an organism. In the present study, we examined the ionomic responses to nutrient deficiency in wheat, maize, sunflower, and soybean. Plants were cultivated in the long-term fertilizer experimental field with four fertilizer treatments, complete fertilization (+NPK), without nitrogen (-N), without phosphorus (-P), and without potassium (-K). Accumulation of various elements in leaves and roots decreased and increased by -N and -K treatments, respectively. In leaves with -P treatment, only sunflower showed increased accumulation of several metal elements including chromium, cadmium, nickel, cobalt, aluminum, copper, barium, and iron. The specific phosphorus acquisition

mechanism(s) in sunflower roots (rhizosphere) may be related to these increases. Among the elements whose accumulation changed due to the treatment, we focused on molybdenum because its accumulation in leaves remarkably increased under nitrogen deficiency in non-leguminous species. We conducted additional experiments using wheat to examine the relationship between nitrogen deficiency and molybdenum. Even in different growth conditions such as pot soil culture, hydroponics, and aseptic culture, the nitrogen deficiency-induced increase of molybdenum accumulation were always observed in leaves of wheat. Because molybdenum supply to soils enhanced the growth of wheat under nitrogen deficiency, increased molybdenum uptake might be involved in the adaptation mechanisms of wheat to nitrogen deficiency. Wheat under nitrogen deficiency accumulated more molybdenum in lower leaves. Moreover, molybdenum application increased and decreased nitrogen concentration in lower and upper leaves of wheat grown under nitrogen deficiency, respectively. These results suggest that molybdenum might affect the nitrogen translocation from older to younger leaves.

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POTENTIAL FOR PLANT GROWTH PROMOTION OF RHIZOBACTERIA ASSOCIATED WITH SALIX AND ELEOCHARIS PLANTS GROWING IN PETROCHEMICAL CONTAMINATED SOILS

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Phytoremediation, a method of using plant holobiont to degrade the contaminants in the environment, is particularly appropriate for organic pollutants. However, the respective contribution of each member within the plant holobiont in the efficiency of phytoremediation is poorly known. Identification of plant-associated bacteria capable of efficiently utilizing these compounds as carbon source and promoting plant growth is a keystone for holobiont engineering and to improve the efficiency of phytoremediation. In this study, we sampled the rhizosphere and the adjacent bulk soils of *Salix* and *Eleocharis* plants from a petrochemical-contaminated area in Varennes, Québec. The aims of this work were (i) to isolate and identify indigenous bacteria inhabiting these contaminated soils; (ii) to assess the isolates ability to grow in the presence of alkanes and polycyclic aromatic hydrocarbons (PAHs) as the sole carbon source, and (iii) to characterize their plant-growth-promoting (PGP) traits determined by : 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, indole-3-acetic acid (IAA) production, nitrogen fixation, phosphate solubilisation and siderophores production. A total of 420 morphologically different bacterial strains were isolated and identified using 16S rRNA gene sequencing. Identified isolates belong to *Arthrobacter*, *Burkholderia*, *Pseudomonas*, *Ralstonia*, *Variovorax* and to other bacterial clades. Forty seven percent of the isolates utilized n-hexadecane as a sole carbon source and many were able to grow on PAHs. Many bacterial isolates showed at least one PGP characteristic. The best hydrocarbon degraders and plant growth-promoting rhizobacteria were selected for evaluating the phytoremediation efficiency under gnotobiotic and hydroponics systems. The results of this research will help improve the bioremediation of petrochemical contaminated fields.

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LAALMT1 ENCODES A VASCULAR VESSEL LOCALIZED MALATE AND CHLORIDE TRANSPORTER

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Lupinus albus (white lupin) massively releases citrate and malate when grown in P-deprived conditions to mobilize sparingly soluble phosphates. The members of the aluminum-activated malate transporter (ALMT) family are candidates for encoding efflux transporters, as these have been reported to transport malate and/or inorganic anions across plant membranes. *LaALMT1* expression, however, was unaffected by –P stress and similarly expressed in different zones of clusters. However, expression decreased in the pre-emergence zone of clusters under –N, –Fe and –Mn stress, while +Al repressed *LaALMT1* in juvenile clusters. A translational fusion of the native promoter with the β -glucuronidase (GUS) reporter gene identified expression in the vascular bundles. Malate release from roots was low in –P, but elevated in +Al, together with citrate. Both organic anions increased in the xylem sap in –P and +Al. Electrophysiological analysis and a ¹³C-labeled citrated efflux assay in *Xenopus laevis* oocytes demonstrated that *LaALMT1* mediated large inward-directed currents that correspond to the efflux of anions. Both, malate and chloride were transported in an Al-independent manner, while a small citrate transport may also occur. Further physiological function characterization of *LaALMT1* was done by generating composite lupins with transgenic roots using CRISPR-Cas9 technology. *almt1* knock-out roots were generated with high efficiency and compared with the wild type, *almt1* mutants exhibited slightly altered organic anion concentrations in root exudates. We conclude that *LaALMT1* functions as a release transporter or channel with minor function in the release of organic and inorganic ions from the root, that may release malate into the xylem and chelate metals for further translocation to the shoots.

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ISOLATION OF A 1,4-DIOXANE DEGRADING PSEUDOMONAS SPP. FROM THE RHIZOSPHERE OF POPLARS WITH BIOREMEDIATION POTENTIAL

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The organic compound 1,4-dioxane is an emerging groundwater pollutant. Removal of 1,4-dioxane from water is very challenging due to the low Henry constant ($4.88 \cdot 10^{-6} \text{ atm} \cdot \text{m}^3 \cdot \text{mol}^{-1}$ at 20 °C), high boiling point (101.1 °C), low vapor pressure (30 mmHg at 20°C) and the high solubility in water ($\log K_{ow} = -0.27$). Moreover, contaminations of this compound are very likely to spread, because of its high stability in water and poor adhesion to soil particles. At first 1,4-dioxane was considered to be non-biodegradable. However the last two and a half decades microorganisms have been found that can degrade this compound. Here we report the isolation of a novel 1,4-dioxane degrader from the rhizosphere of poplar trees on an industrial site contaminated with 1,4-dioxane. Microcosms of the rhizosphere of poplar trees in the plume zone and the soil from the contaminated source zone were prepared. Subsequently bacteria were enriched and selected for growth on 1,4-dioxane. A total of 16 strains were isolated capable of growing on 1,4-dioxane as the sole carbon

source, 14 of these strains originated from the rhizosphere and only 2 from the source zone. The three fastest growing strains were further characterized and all originated from the poplar rhizosphere. This suggests the rhizosphere of poplars trees creates a favorable environment for bacteria to degrade 1,4-dioxane. The fastest degrader was identified by 16s RNA sequencing to be a pseudomonas species, this is to our knowledge the first report of a pseudomonas species capable of metabolically degrading the pollutant 1,4-dioxane. This bacterium has the potential to be applied for the bioremediation of 1,4-dioxane polluted soils and groundwater. Our research highlights the potential of rhizosphere bacteria for remediation of various organic pollutants.

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IMPACT OF LONG-TERM N AND P FERTILIZATION ON SOIL-MICROBIAL MEDIATED P CYCLING PROCESSES

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The development of sustainable agriculture practices that aim to improve soil nutrient availability and cycling faces numerous challenges with long-term use of N and P fertilization. The objective of this study was to determine how long-term N and P fertilization practices impact soil-microbial mediated P cycling processes in bulk and rhizosphere soil. This study was based in a long-term field trial of continuous wheat (*Triticum aestivum* L.) since 1967 and included four treatments: N fertilization and no P (+N-P), N and P fertilization (+N+P), P fertilization and no N (-N+P), and no fertilization (-N-P). Soil samples were collected prior to seeding (April), every two weeks during the growing season (May to August), and after harvest (September) to evaluate temporal variability of various soil biological parameters linked to P cycling processes. These included enzyme activity assays, microbial biomass, P cycling functional gene abundance, and bacterial (16S) and fungal (ITS) communities. We observed that long-term N fertilization significantly increased acid phosphomonoesterase activity, and decreased alkaline phosphomonoesterase and phosphodiesterase activity. P fertilization only had a significant effect on enzyme activity in plots that did not receive long-term N fertilization (i.e., -N-P and -N+P). N fertilization inhibited β -glucosidase activity and increased N-acetyl- β -D-glucosaminide activity. Overall, similar trends were observed in the bulk and rhizosphere soil, but higher levels of enzyme activity in the rhizosphere soil were particularly evident during the peak growing season (July and August). Long term N and P fertilization can influence soil P cycling through changes in related enzyme activities and further evaluation of the additional soil biological parameters will provide better insight into the impact of these crop management practices on soil microbial mediated aspects of P cycling.

P.198**RHIZOSPHERE MICROORGANISMS IN CONTINUOUS CROPPING TOMATO FIELD IDENTIFIED BY AMPLICON-BASED METAGENOMICS AS MINOR PATHOGEN CANDIDATES**Y Gao¹, S Li¹, R Guo¹, X Lu¹¹*Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China*

Microorganisms play pivotal roles in keeping plants and soil healthy. Beneficial and pathogenic microorganisms colonizing plant rhizosphere are well studied. However, it is noteworthy that some rhizosphere microbes are considered as minor pathogens which contribute to runtishness, stuntedness, leaf chlorosis and yield reduction without obvious symptoms on plants or with symptoms under specific environmental conditions. Our previous investigation indicated that continuous cropping is closely related to the increase of minor pathogens. Herein, high-throughput amplicon sequencing method was used to characterize the rhizosphere microbial communities of continuous cropping tomato fields. A total of 22 soil samples were collected from the greenhouse of Liaoning, China, and classified into six groups according to the continuous cropping periods (0, 1~3, 5~7, 10~15, and 20~30 years). Bacterial and fungal communities were determined by high throughput Illumina Miseq300 and Miseq250 sequencing of 16S rRNA and ITS1 genes, respectively. Through partial least square discriminant analysis, substantial variations were observed among the six groups with different cropping time, especially among the 0-year group and other groups and below 3-year groups and over 3-year groups. Significant differences were also detected in microbial species between groups (one-way ANOVA analysis, $p < 0.05$). The relative abundance of three fungal families (*Olpidium*, *Penicillium*, and *Fusarium*) and five bacterial genera (*Streptomyces*, *Luteimonas*, *Chitinophagaceae*, *Flavobacterium* and *Arachidicoccus*) showed increasing trends along with the continuous cropping years. Based on the previous studies of pathogenic microorganisms, three cultivable fungal genera (*Olpidium*, *Penicillium*, and *Fusarium*) and three bacterial families (*Xanthomonadaceae*, *Streptomycetaceae*, and *Flavobacteriaceae*) were considered as minor pathogen candidates. This study will benefit our understanding of the microbial communities in continuous cropping system and their roles in plant sub-health, especially the stuntedness and yield reduction. Further research will be conducted to isolate the candidate pathogens and verify their biological functions.

P.199**BELOWGROUND BATTLES IN BRASSICACEAE. OPTIMIZING CHEMICAL DEFENSE ALLOCATION UPON CABBAGE ROOT FLY FEEDING**NM van Dam^{1,2}, R Sontowski^{1,2}, N Gorringer³, A Touw^{1,2}, T Tsunoda^{1,4}¹*German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany*²*Institute for Biodiversity, Friedrich-Schiller-University Jena, Jena, Germany*³*Cardiff University, Cardiff, United Kingdom*⁴*Faculty of Agriculture, Shinshu University, Nagano, Japan*

Optimal defence theory predicts that the most valuable and the most vulnerable plant organs should have the highest levels of chemical defences. The optimal defence theory has been mostly applied to aboveground defence allocation patterns. Recently, we found that the principles of the optimal defence theory also apply to

roots. Both constitutive and inducible glucosinolates, the main chemical defences in Brassicaceae, are predominantly allocated to taproots. This is in line with the optimal defence theory, because we found that herbivory on taproots decreased plant performance more than herbivory on fine roots. Here we present our latest research on the underlying molecular and chemical mechanisms of belowground defence allocation. Using *Brassica rapa* and root feeding larvae of cabbage specialist *Delia radicum*, we addressed the following questions: 1) do *Delia*-induced allocation patterns concur with changes in local and systemic glucosinolate biosynthesis and transport gene expression? 2) Do different classes of root glucosinolates respond similarly? 3) Do *Delia*-induced allocation patterns differ among *B. rapa* accessions with high and low constitutive glucosinolate levels?

Our results show that both glucosinolate synthesis and transport gene expression increase locally in taproots of *Delia* infested plants. Especially indole glucosinolate levels and the expression of an indole biosynthesis gene increased rapidly, independent of whether the accession had high or low constitutive glucosinolate levels. At the same time aliphatic glucosinolate levels in taproots decreased. In the fine roots, on which *Delia* does not feed, glucosinolate and gene expression remained mostly constant. This means that *B. rapa* plants respond to root feeding by inducing specific classes of glucosinolates while downregulating the synthesis of others. Moreover, they prioritize local responses to the more valuable taproots as predicted by the optimal defence theory.

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IN SITU CULTIVATION REVEALS A HIGH PORTION OF BENEFICIAL CULTURABLE BACTERIA IN THE RHIZOBIOME OF WHEAT PLANTS (TRITICUM AESTIVUM) GROWN IN CHILEAN ANDISOL

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The plant rhizobiome harbors a great variety of bacteria known as plant growth-promoting rhizobacteria (PGPR) that promote the growth and fitness of crops. However, only a minor portion ($\leq 1\%$) of environmental bacteria, including PGPR, can be cultured by traditional culture methods. Recent advances in in situ cultivation methods have opened a window to explore the diversity of environmental bacteria. Here, we explored the total and culturable bacterial communities in the rhizobiome of wheat plants grown in Chilean Andisol using high-throughput DNA sequencing (HTS) and in situ cultivation with microwell chambers (MWCs), respectively. Bacterial communities and isolated strains were characterized based on 16S rRNA gene sequencing, with consideration of their potential as PGPR. Proteobacteria (29-39%), Acidobacteria (17%), Actinobacteria (11-15%) and Bacteroidetes (5-12%) were the most abundant and active bacterial taxa in the rhizosphere samples, as revealed by HTS. A total of 206 isolates were recovered from the MWCs, and the most abundant phyla were Proteobacteria (70.4%), Firmicutes (24%), Actinobacteria (4%) and Bacteroidetes (1.5%). At the genus level, the majority of isolates belonged to *Pseudomonas* (45 isolates), followed by *Bacillus* (40 isolates),

Stenotrophomonas (35 isolates), Delftia (16 isolates), and Herbaspirillum (13 isolates). The use of MWCs also allowed the isolation of bacteria commonly considered rare taxa (e.g., Lelliottia, Rhodococcus, Micrococcus, Variovorax, and Bosea). In addition, a high proportion (82%) of isolates (169) showed high similarity with beneficial bacteria. A high portion of 26 selected strains (one of each genus) also showed PGPR traits, such as siderophore production (58%), auxin production (50%), nitrogen fixation (46%) and phosphorus solubilization (19%). In situ cultivation in MWCs represents an attractive strategy to isolate an increased diversity of potential PGPR for cereal crops, such as wheat.

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DIFFERENTIAL TRANSCRIPTOMIC REPROGRAMMING IN ARABIDOPSIS ROOTS IN RESPONSE TO FOLIAR INFECTION BY DISTINCT PATHOGENS

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Plants can adjust their root microbiome upon pathogen infection and specifically recruit disease resistance-inducing microbes. As a result, infected plants create a soil-borne legacy that confers enhanced protection against the pathogen in a second population of plants growing in the same soil. This has been demonstrated in Arabidopsis for both *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* infections. In this study, we wanted to shed light on the genetic mechanism by which plants recruit beneficial microbes in response to attack. Moreover, we compared the plants responses to pathogens with distinct lifestyles. To this end, we analyzed root transcriptomic responses of *Arabidopsis thaliana* plants inoculated with *Hyaloperonospora arabidopsidis*, *Phytophthora capsici*, *Pseudomonas syringae* pv. *tomato* DC3000 or *Botrytis cinerea* in the leaves. Root tissues were harvested and the root transcriptome of infected and control plants was analyzed. Changes in the root transcriptome are analyzed with the aim to identify differentially expressed genes and, through subsequent gene set enrichment analysis, biosynthetic pathways or other processes that are likely involved in the creation of soil-borne legacies.

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RHIZOBACTERIAL VOLATILES; CRITICAL COMPONENTS OF A SUSTAINABLE TOOLKIT FOR IMPROVING PLANT QUALITY AND GROWTH

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The study of volatile organic compounds from bacteria and their impact on plant growth and quality is a relatively new field of research less than two decades old. During that time a number of key compounds have been identified that are known plant growth-promoters and/or have antifungal activity. During the course of our research over the past ten years, working firstly in the Andes in South America (VALORAM FP7-funded project) and more recently looking at rhizobacterial isolates from both organic and conventionally managed potato fields in the South of Ireland, we have identified key microbial volatile organic compounds (mVOCS) that impact the plant in a number of different ways (dry weight, response to biotic and abiotic stresses and altered gene expression). Bacteria were isolated from soil using standard techniques and sequenced using 16s ribosomal RNA sequencing. Sequences were identified using multiple sequence alignment tools. The isolates were identified as mainly *Bacillus*, *Serratia* and *Lysinibacillus*. We have developed a novel technique for determining the impact of mVOCS on microplants under in vitro conditions. We established microplants of potato in vitro in Microboxes[®] and tested rhizobacterial mVOCS for impact on plant growth and antifungal capability. In a separate experiment, eight rhizobacterial isolates were grown on three different media types for 24 hours (MRVP, TSB and MS) and volatile signatures detected using Solid Phase Microextraction Gas Chromatography Mass Spectrometry. Media composition did have an impact on the type of volatile produced with isolates grown on TSB producing the greatest variety of compounds. Several rhizobacterial isolates produced known plant growth-promoters such as 2,3-butanediol and 2-butanone and antifungal compounds such as dodecane. Our study highlights the importance of understanding the microbial volatilome. We suggest that rhizobacterial volatiles are critical components of a sustainable toolkit for improving plant quality and growth.