

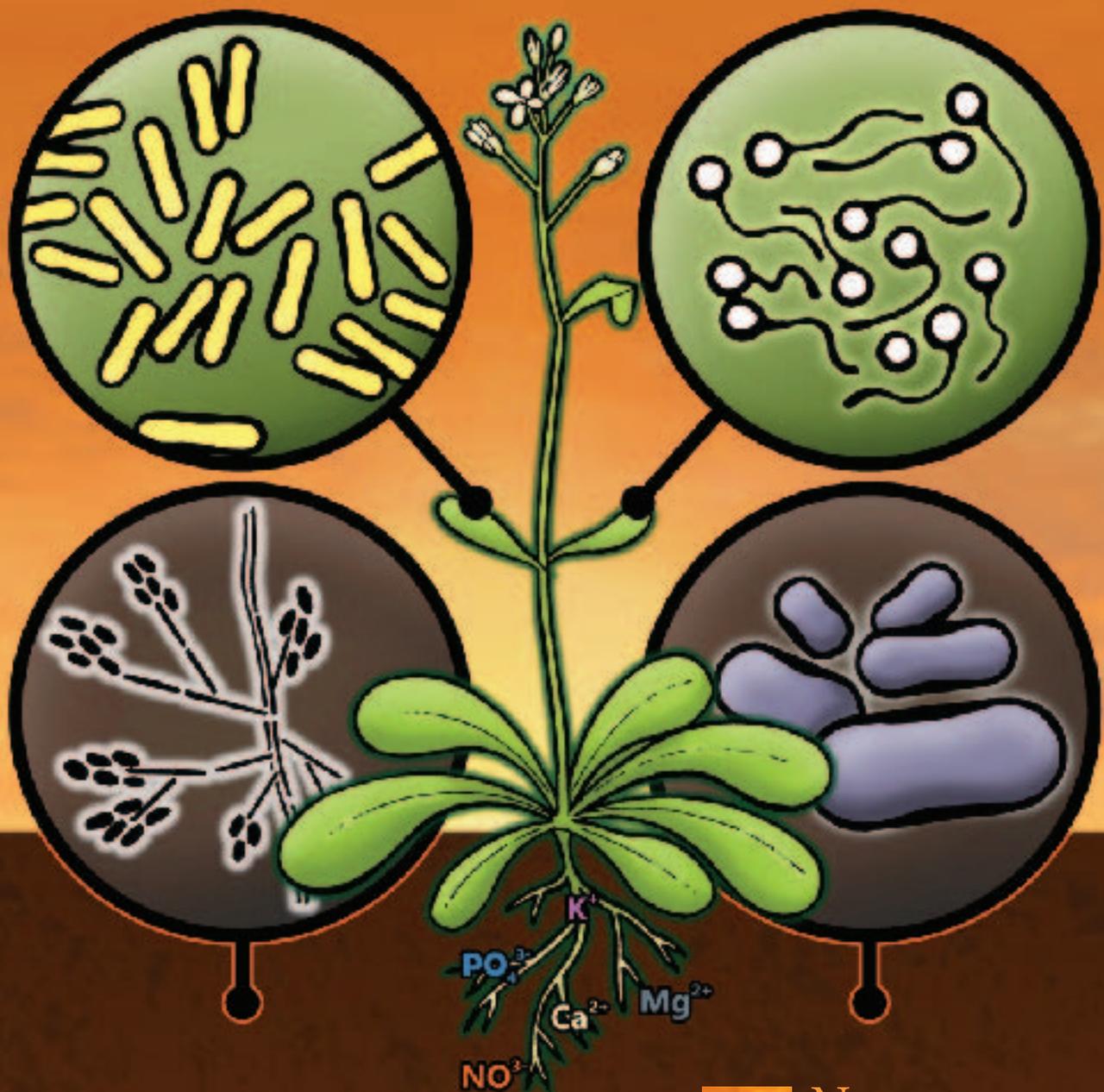
28th New Phytologist Symposium

# Functions and ecology of the plant microbiome

18–21 May 2012

Aldemar Hotel, Rhodes, Greece

Programme, abstracts and participants



New  
Phytologist

# 28th New Phytologist Symposium

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## Functions and ecology of the plant microbiome

Aldemar Hotel, Rhodes, Greece

### Scientific Organizing Committee

Jeff Dangl (*University of North Carolina, Chapel Hill, USA*)

Paul Schulze-Lefert (*Max Planck Institute for Plant Breeding Research, Cologne, Germany*)

### New Phytologist Organization

Helen Pinfield-Wells (*Deputy Managing Editor*)

Jill Brooke (*Journal & Finance Administrator, New Phytologist*)

Holly Slater (*Managing Editor*)

### Acknowledgements

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### New Phytologist Trust

The New Phytologist Trust is a non-profit-making organization dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at [www.newphytologist.org](http://www.newphytologist.org)

Programme, abstracts and participant list compiled by Jill Brooke

‘Functions and ecology of the plant microbiome’

illustration by A.P.P.S., Lancaster, UK

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# Information for Delegates

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## Meeting Location

The 28<sup>th</sup> New Phytologist Symposium will be held at the ALDEMAR Paradise Mare Hotel, Rhodes, Greece 18-21 May 2012. The main meeting will take place in the Rhodes & Patmos rooms.

## Catering

As all inclusive guests at the Aldemar Paradise Mare hotel you should wear your wrist band at all times.

**Coffee breaks** will be served in either the Kos & Karpathos rooms or the Conference center veranda. This is indicated in the programme.

**Breakfast** can be taken in the main restaurant 'Symposio'.

Continental Breakfast (cold options available only) 07:00-07:30

American Style Buffet Breakfast (hot options available) 07:30-10:00

**Lunch** can be taken in any of the following restaurants; 'La pergola' Italian restaurant 12:00-16:00, 'Dionysos' restaurant 12:00-15:30 & 'Albatros' Beach restaurant 12:00-16:00

**Dinner** can be taken in either of the following restaurants; Main restaurant 'Symposio' 18:30-21:15, 'La pergola' Italian restaurant 18:30-21:15

To dine in the Chinese Restaurant 'Goldern Palm' delegates should make a reservation at this theme restaurant.

There is also a variety of bars/restaurants which are within the Aldemar Paradise Mare resort which can be used by delegates; Beach restaurant and Greek Tavern 'Thalassa' - Delegates may eat at both restaurants, Main bar 'Chevalier' 17:00-01:00, Cocktail bar 'Eva' Open until 00:00, Pool bar 'Delfinia' 10:00-18:00, Night Club 'Safari' Open until 00:00. From 00:00-02:00 beverages will be charged for.

**Conference dinner** on Sunday evening will commence at 20:00 in 'Dionysos' Restaurant.

## Posters

If you have submitted a poster abstract to share your research with the community this should be A0 in size and portrait in orientation. Please display your poster as soon as possible on the 18<sup>th</sup> May, on the numbered board which corresponds with the number your poster abstract has been allocated in the abstract book. Pins will be available. Please remove all posters by 14:00 on Monday 21st May.

Posters will be open for viewing throughout the symposium and will be located in the rooms (Kos & Karpathos) next to the main meeting room. Two specific sessions on Friday 18:30-20:00 and Saturday 18:30-20:00 will also take place.

## Maps

Maps of the conference centre and the hotel complex are provided at the back of this booklet.

## Tour

The tour will take place on Sunday afternoon and will go to Rhodes town to visit the Ancient Acropolis of Rhodes, the Grand Masters' Palace and the Street of Knights. This is open to all delegates who are attending the symposium and there is no additional charge for this. If you are not planning to join the tour please let Helen know.

Please meet in reception at 13:15 and we will be taken to the buses. The tour will involve some walking so please wear comfortable shoes and bring a sun hat! Buses will return us to the Aldemar Paradise Mare around 17:15.

## Internet Access

The hotel offers internet access. This is free of charge within the hotel property.

# Meeting Programme

| Friday 18 <sup>th</sup> May   |  |                                 |
|-------------------------------|--|---------------------------------|
| 14:00-17:00                   | Registration   | Conference/Olympic Hall 1 Lobby |
| 17:15-17:30                   | Welcome and Introductions<br>Jeff Dangl and Paul Schulze-Lefert  | Rhodes & Patmos                 |
| 17:30-18:30                   | Keynote plenary - The plant microbiome: Uniquely green microbiology<br><i>Jo Handelsman, Yale University, CT, USA</i>                    |                                 |
| 18:30-20:00                   | Posters and Welcome Reception  | Kos & Karpathos                 |
| 20:00                         | Dinner   |                                 |
| Saturday 19 <sup>th</sup> May |  |                                 |
| 8:50-9:00                     | Announcements  | Rhodes & Patmos                 |
| Session 1                     | <b>Rhizosphere microbiology and biochemical cycling</b><br><i>Chair: Jo Handelsman</i>   | Rhodes & Patmos                 |
| 9:00-09:30                    | Metagenomics of the rhizosphere microbiome: understanding the interplay between the good, the bad and the ugly<br><i>Jos Raaijmakers</i> |                                 |
| 9:30-10:00                    | Root physiology and rhizosphere ecology: pH is the clue<br><i>Philippe Hinsinger</i>   |                                 |
| 10:00-10:30                   | Carbon trading at the soil-root interface, the case of the Oloton corn from Oaxaca<br><i>Pablo Andres Zamora</i>                         |                                 |
| 10:30-11:00                   | Tea/coffee break   | Kos & Karpathos                 |
| 11:00-11:15                   | Selected poster talk P19: The core and pan-genome of serpentine-adapted <i>Mesorhizobia</i><br><i>Maren Friesen</i>                      |                                 |
| 11:15-11:45                   | Microbial ecology of the rhizosphere<br><i>Wafa Achouak</i>  |                                 |
| 11:45-12:00                   | Selected poster talk P78: Selection of plant growth promoting rhizobacteria by plant model species<br><i>Andrzej Tkacz</i>               |                                 |
| 12:00-12:30                   | Discussion of Session 1  |                                 |
| 12:30-13:45                   | Lunch  |                                 |
| Session 2                     | <b>Soil microbial communities</b><br><i>Chair: Jeffrey Bennetzen</i>   | Rhodes & Patmos                 |
| 13:45-14:15                   | Effect of potato cultivar type on plant-associated bacterial communities as determined by pyrosequencing<br><i>Jan Dirk van Elsas</i>    |                                 |

|                                   |   |                           |
|-----------------------------------|---|---------------------------|
| 14:15-14:30                       | Selected poster talk P2: Characterization of native root fungal endophytes and their impact on tomato plant growth and health<br><i>Diana Andrade-Linares</i> |                           |
| 14:30-15:00                       | Induced systemic resistance and the rhizosphere microbiome<br><i>Peter Bakker</i>   |                           |
| 15:00-15:30                       | Structure and function of soil microbial communities<br><i>Ralf Conrad</i>  |                           |
| 15:30-16:00                       | Tea/coffee break  | Conference Center Veranda |
| Session 3                         | Phyllosphere and endophytic microbial communities<br><i>Chair: Peter Bakker</i>   | Rhodes & Patmos           |
| 16:00-16:30                       | Host control and organization of the <i>Arabidopsis</i> microbiome<br><i>Jeff Dangl</i>   |                           |
| 16:30-16:45                       | Selected poster talk P35: The role of innate immunity in shaping the <i>Arabidopsis</i> phyllosphere microbiome<br><i>James Kremer</i>                        |                           |
| 16:45-17:15                       | Identifying bacterial traits mediating epiphytic fitness<br><i>Stephen Lindow</i>   |                           |
| 17:15-17:45                       | Microbial life of indigenous phyllosphere bacteria<br><i>Julia Vorholt</i>  |                           |
| 17:45-18:30                       | Discussion of Sessions 2 and 3  |                           |
| 18:30-20:00                       | Poster session  | Kos & Karpathos           |
| 20:00                             | Dinner  |                           |
| <b>Sunday 20<sup>th</sup> May</b> |   |                           |
| 8:50-9:00                         | Announcements   | Rhodes & Patmos           |
| Session 4                         | Genetic control and selection of plant microbiomes<br><i>Chair: Julia Vorholt</i>   | Rhodes & Patmos           |
| 9:00-9:30                         | Plant-bacteria interaction in the rhizosphere based on signalling compounds related to bacterial quorum sensing<br><i>Anton Hartmann</i>                      |                           |
| 9:30-10:00                        | Genetic control of maize, <i>Sarracenia</i> , <i>Setaria</i> and switchgrass microbiomes<br><i>Jeffrey Bennetzen</i>  |                           |
| 10:00-10:30                       | Organization and control of the rice microbiome<br><i>Venkatesan Sudaresan</i>  |                           |
| 10:30-11:00                       | Tea/ Coffee break   | Kos & Karpathos           |
| 11:00-11:15                       | Selected poster talk P13: Impact of jasmonate signalling on the plant-soil microbiome<br><i>Lilia Carvalhais</i>  |                           |

|                                   |   |                           |
|-----------------------------------|---|---------------------------|
| 11:15-11:45                       | Structure and host control of the <i>Arabidopsis</i> root microflora<br><i>Paul Schulze-Lefert</i>  |                           |
| 11:45-12:15                       | Discussion of Session 4   |                           |
| 12:15-13:15                       | Lunch   |                           |
| 13:15                             | Buses depart for a tour of Rhodes town<br><i>Ancient Acropolis of Rhodes - Grand Masters' Palace - Street of Knights</i>  |                           |
| 17:15                             | Buses arrive back at Aldemar Paradise Mare Hotel  |                           |
| 18:30-19:30                       | Keynote plenary: Dissecting a 'coastal marine microbiome': Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom<br><i>Rudolf Amann, MPI for Marine Microbiology, Bremen, Germany</i> |                           |
| 19:30                             | Drinks Reception  |                           |
| 20:00                             | 'Night of Knights' Conference Dinner  | Dionysos Restaurant       |
| <b>Monday 21<sup>st</sup> May</b> |   |                           |
| 8:50-9:00                         | Announcements   | Rhodes & Patmos           |
| Session 5:                        | Characterization and ecology of plant microbiota<br><i>Chair: Jan Dirk van Elsas</i>  | Rhodes & Patmos           |
| 9:00-9:30                         | Beneficial interactions of endophytic bacteria on their host plants<br><i>Daniel van der Lelie</i>  |                           |
| 9:30-10:00                        | Micro-managing sustainability: Ecology of the <i>Miscanthus</i> -associated microbiome<br><i>Angela Kent</i>  |                           |
| 10:00-10:30                       | Factors shaping the microbial community within <i>A. thaliana</i><br><i>Joy Bergelson</i>   |                           |
| 10:30-11:30                       | Tea/Coffee break  | Conference Center Veranda |
| 11:00-11:15                       | Selected poster talk P3: Characterization of the root mycobiome in the perennial Brassicaceae species <i>Arabidopsis thaliana</i><br><i>Marcel Bucher</i>   |                           |
| 11:15-11:45                       | Culture-independent community profiling and directed isolation of the sugarcane rhizosphere<br><i>Phil Hugenholtz</i>   |                           |
| 11:45-12:15                       | Plant microbe interactions in the <i>Populus</i> rhizosphere<br><i>Jerry Tuskan</i>   |                           |
| 12:15-13:00                       | Discussion and Symposium summary  |                           |
| 13:00-14:00                       | Lunch   |                           |
| 14:00                             | Close of Symposium  |                           |

# Speaker Abstracts

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\* S=speaker abstract; P=poster abstract

|                        |                               |
|------------------------|-------------------------------|
| Achouak, Wafa          | S1.4                          |
| Amann, Rudolph         | Keynote Plenary 2             |
| Bakker, Peter          | S2.2, P6, P14                 |
| Bennetzen, Jeffrey     | S4.2                          |
| Bergelson, Joy         | S5.3                          |
| Conrad, Ralph          | S2.3                          |
| Dangl, Jeff            | S3.1, P31, P38, P42, P43, P90 |
| Handelsman, Jo         | Keynote Plenary 1             |
| Hartmann, Anton        | S4.1, P93                     |
| Hinsinger, Philippe    | S1.2                          |
| Hughenoltz, Phil       | S3.1, S5.4                    |
| Kent, Angela           | S5.2, P39                     |
| Lindow, Stephen        | S3.2                          |
| Raaijmakers, Jos       | S1.1, P14, P37, P48           |
| Schulze-Lefert, Paul   | S4.4, P4, P9, P65, P70        |
| Sundaresan, Venkatesan | S4.3, P10                     |
| Tuskan, Jerry          | S5.5                          |
| van der Lelie, Daniel  | S5.1                          |
| van Elsas, Jan Dirk    | S2.1, P53                     |
| Vorholt, Julia         | S3.3, P8, P85                 |
| Zamora, Pablo          | S1.3                          |

## The plant microbiome: Uniquely green microbiology

# Keynote Plenary 1

17:30-18:30

Jo Handelsman

Plant biologists recognized the role of microorganisms in host health long before the human microbiome acquired celebrity status. In 1904, Hiltner recognized that the dense, active community of microorganisms on and around roots act as an extension of the plant, designating this region ‘the rhizosphere’. For decades, epiphytic and endophytic bacteria have received attention through the study of biological control (suppression of plant disease with microbial inoculants), nitrogen fixation, disease suppressive soils, and fermentation of plant material into food for human consumption. During a century when microbiology of human beings veered toward the reductionist, favoring the one-microbe-one-disease model of host-microbe interactions, study of plant-microbe interactions continued to include the dynamic and complex community that encases the plant. Despite the historical commitment and attention to the plant microbiome, its study has not kept pace with the recent surge of research activity in the human microbiome. The injection of massive funding and the celebration of the marriage of systems-level analysis with reductionist approaches have catapulted the human microbiome into one of the most visible and active spheres of biological research. The plant microbiome is worthy of the same attention for its value to fundamental understanding of biology and stability of food production. Every plant organ deserves to be the subject of a comprehensive census of its microbial members. Massive efforts in sequence- and function-based metagenomic analyses should be launched to understand the contributions of microbial communities to plant health. Just as study of the human microbiome has produced surprises about the variety and abundance of human conditions influenced by microbial communities (diabetes, obesity, sleep cycles, and depression), thorough analysis of the plant microbiome is likely to be surfeit with surprises that will delight the curious mind and enable vital advances in crop health. This presentation will unite traditional study of the plant microbiome with recent advances using molecular approaches, and it will examine the aspects of future study that are especially tractable in plants.

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## Session 1: Rhizosphere microbiology and biochemical cycling

Chair: Jo Handelsman

### Metagenomics of the rhizosphere microbiome: understanding the interplay between the good, the bad and the ugly

Disease-suppressive soils are exceptional ecosystems in which beneficial microorganisms guard plants from infections by soil-borne pathogens. For most suppressive soils, the microorganisms and mechanisms involved in disease suppression are not known to date. Here, PhyloChip-based metagenomics of the rhizosphere microbiome was coupled with culture-dependent functional analyses to identify bacterial taxa and mechanisms involved in soil suppressiveness to the fungal root pathogen *Rhizoctonia solani*. The metagenomic analyses led to the identification of diverse bacterial and archaeal taxa and specifically pointed to the Proteobacteria, Firmicutes and Actinobacteria as the most dynamic groups associated with disease suppression. Targeted isolation and functional analyses led to the identification of specific members of the  $\gamma$ -Proteobacteria that produce chlorinated antifungal peptides that inhibit hyphal growth of *R. solani*. Next to the beneficial rhizobacteria, metagenomic analysis also revealed the presence of several opportunistic and true human pathogenic bacteria in the rhizosphere microbiome, including Staphylococcus, Salmonella, Clostridium and Vibrio species. Preliminary analysis indicated that several of these bacterial taxa are more abundant in the rhizosphere of sugar beet plants grown in the conducive soil than in suppressive soil. The interplay between the good, the bad and the ugly in the rhizosphere microbiome will be presented.

# S1.1

9:00-09:30

<sup>1</sup>Jos M. Raaijmakers,  
<sup>1,2</sup>Rodrigo Mendes

<sup>1</sup>Laboratory of Phytopathology, Wageningen University, Wageningen, the Netherlands; <sup>2</sup>Embrapa Environment, Jaguariuna, Brazil

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## Root physiology and rhizosphere ecology: pH is the clue

# S1.2

9:30-10:00

**Philippe Hinsinger**

Roots can considerably alter soil pH - up to several units - over short spatial and temporal scales as a result, primarily, of their nutrition (cation/anion balance across the root/soil interface) and, in some instances, respiration. We will briefly review how these root-induced pH changes have a dramatic effect on key biogeochemical processes such as: (i) dissolution/precipitation of soil minerals and (ii) adsorption/desorption of ions, and hence bioavailability of either anions (e.g. phosphate) or cations (e.g. copper). Further understanding the resulting, rather unique rhizosphere biogeochemistry is thus pivotal to quantifying the driving role of higher plants in ecosystem services, especially support services such as soil formation and nutrient cycling. In addition, soil pH has been recently shown to be a major ecosystem property governing the biogeography of soil microbial communities across spatial scales, from continental to local (plot) scales. Here we report the first evidence of the role of root-induced pH changes on the distribution of microbial communities at a centimetric scale, in the rhizosphere of ectomycorrhizal plants. This finding challenges the view of a carbon-based rhizosphere effect and suggests that, beside rhizodeposition of carbon compounds, rhizosphere pH may be the clue for shaping microbial ecology in the root environment.

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## Carbon trading at the soil-root interface, the case of the Oloton corn from Oaxaca

# S1.3

10:00-10:30

Adaptations of plants to the environment require a series of successful strategies ranging from signaling responses to morphological modification. *Zea mays* cv. Oloton is a very specific landrace of maize growing historically in nutrient deficient soils in an isolated village near Oaxaca, Mexico; the center of origin and domestication of corn. These plants can grow up to five meters tall, generate extensive biomass, develop up to eight aerial roots and secrete, from these aerial roots, large volumes of mucilage. The mucilage is comprised of unique fucosylated complex carbohydrates that are secreted most abundantly during a six to eight week period that is synchronized with seed filling. To understand the role of mucilage secretion we conducted sugar profiling and metabolome profiling. Our results suggest that this trait is a strategy to promote plant defense and nutrient acquisition.

**<sup>1</sup>Pablo Zamora, <sup>1-2</sup>  
**Howard-Yana  
Shapiro, <sup>1</sup> Alan  
Bennett****

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11:15-11:45

**Wafa Achouak**

Plants exert a major influence on microbial communities through the release of signalling molecules and a range of organic compounds, such as root exudates. The amount and composition of root exudates vary between plant species, and thus their influence on microbial community structure in the rhizosphere is also presumed to differ. Plants undeniably shape the microbial community structure in the vicinity of roots, and this impact is greatest within the root compartment. Up to 20% of the carbon capital fixed by plants through photosynthesis is invested belowground as root exudates. In return, plants benefit from the microbial turnover of root exudates, and other soil organic and inorganic matter, which releases nutrients and enhances soil structure. The recently developed “omic technologies” enable investigations into the molecular basis sustaining the establishment of beneficial plant-microbial interactions in the rhizosphere. Further investigation into these ‘specific’ interactions will benefit our knowledge and appreciation of this intriguingly wide diversity of plant-bacteria molecular dialogues.

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## Session 2: Soil microbial communities

*Chair: Jeffrey Bennetzen*

### Effect of potato cultivar type on plant-associated bacterial communities as determined by pyrosequencing

# S2.1

13:45-14:15

Plants selectively attract particular soil microorganisms, in particular the consumers of root-excreted compounds. However, we lack information as to what extent cultivar type and/or growth stage affect the selective process. In this study, we applied DNA-based pyrosequencing to characterize the structure of the bacterial communities in a field cropped with potato. Thus, the microbiota in the rhizospheres of six cultivars, denoted Aveka, Aventura, Karnico, Modena, Premiere and Desiree, at three growth stages (young, flowering and senescence) was examined, in addition to corresponding bulk soils. Around 300,000 filtered and cured sequences were obtained (5,700 to 38,000 per sample). Across all samples, rank abundance distributions best fitted the power law model, which indicates a community composed of a few highly dominant species next to numerous rare species. Grouping of the sequences showed that members of the Actinobacteria and Alphaproteobacteria, next to as-yet-unclassified bacteria, dominated the communities. Other groups that were consistently found, albeit at lower abundance, were Beta-, Gamma- and Deltaproteobacteria and Acidobacteria. Principal components analyses of the relative abundance data revealed that the rhizosphere microbiotas were significantly different from those of the corresponding bulk soils in each growth stage. Furthermore, potato cultivar effects were found in the young plant stage, whereas these became insignificant in the flowering and senescence stages. Besides, an effect of time of season was observed for both rhizosphere and bulk soil communities. The analyzed rhizosphere samples of the potato cultivars were grouped into two groups, in accordance with the allocation of carbon to starch in their tubers, i.e. Aveka, Aventura and Karnico (high) versus Premiere and Desiree (low) and thus replicates per group were established. We conclude that, across all potato cultivars, the young plant stages revealed potato cultivar-dependent bacterial community structures, which disappeared in the flowering and senescence stages. Furthermore, Pseudomonas, Beta-, Alpha- and Deltaproteobacteria flourished under different ecological conditions than Acidobacteria.

**Jan Dirk van Elsas**  
**and Joana Falcao**  
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## Induced systemic resistance and the rhizosphere microbiome

Microbial communities that are associated with plant roots are highly diverse and harbor tens of thousands of bacterial and archaeal species. Several functions sustained by this so-called rhizosphere microbiome are drivers of plant health and include the suppression of infectious diseases. The latter function is prominent in disease suppressive soils. From such soils micro-organisms have been selected that can effectively control soil borne diseases. The mechanisms implicated in disease suppression by these biological control agents include competition for nutrients and space, antibiosis, and induced systemic resistance (ISR). For many biological control agents ISR has been recognized as a mechanism that at least partly explains disease suppression. ISR eliciting traits of biological control agents have been identified, they are diverse and in many cases there is redundancy of ISR elicitors. Implications of ISR on recruitment and functioning of the rhizosphere microbiome are discussed.

## S2.2

14:30-15:00

**Peter A.H.M. Bakker, Roeland L. Berendsen, Corné M.J. Pieterse**

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## Functional analysis of soil microorganisms: transcript analysis of functional genes, stable isotope probing of mRNA, transcriptomics

## S2.3

15:00-15:30

**Ralf Conrad**

A major challenge in soil microbial ecology is to determine which microorganisms are involved in particular biogeochemical processes, such as production and oxidation of methane or ammonia. More generally, it would be interesting to know which of the many different soil microorganisms are active with respect to which process whatever at which time and under which environmental condition. In our department we addressed these questions by analyzing the transcription of genes that code for the enzymes that are key to methane production (*mcrA*), methane oxidation (*pmoA*), and ammonia oxidation (*amoA*) in response to environmental cues, such as soil water status. The transcript analysis was mainly based on quantitative PCR, terminal restriction fragment length analysis, cloning/sequencing, and pyrosequencing. In a step further, we analyzed transcripts of the genes in RNA fractions enriched in  $^{13}\text{C}$  after feeding the soil microorganisms with a particular  $^{13}\text{C}$ -labelled substrate. This allowed the detection of only those microbes that were able to synthesize the transcripts using carbon from this particular substrate. Finally, we analyzed the global transcriptome of all soil microorganisms after separation of mRNA from ribosomal RNA addressing transcription of any functional gene. The advantage and disadvantage of the different approaches will be discussed.

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## Session 3: Phyllosphere and endophytic microbial communities

Chair: Peter Bakker

### Defining the core *Arabidopsis thaliana* root microbiome

The microbiota colonizing the rhizosphere and the endophytic compartment contribute to plant growth, productivity, carbon sequestration, and phytoremediation. Colonization of the root occurs despite a sophisticated plant immune system, suggesting discrimination of mutualists and commensals from pathogens. Genetic principles governing the derivation of host-specific endophyte communities from soil communities are poorly understood. We pyrosequenced the bacterial 16S rRNA gene of >600 *Arabidopsis thaliana* plants to test the hypotheses that the root rhizosphere and endophyte compartment microbiota of plants grown under controlled conditions in natural soils are (i) sufficiently dependent on the host to remain consistent across different soil types and developmental stages, and (ii) sufficiently dependent on host genotype to vary between inbred *Arabidopsis* accessions. We describe different bacterial communities in two geochemically distinct bulk soils, and in rhizosphere and endophyte compartments prepared from roots grown in these soils. The communities in each compartment are strongly influenced by soil type. Endophyte compartments from either soil feature overlapping low-complexity communities that are markedly enriched for Actinobacteria and specific families from other phyla, notably Proteobacteria. Some bacteria vary quantitatively between plants of different developmental stages and genotypes. Our work provides unprecedented rigor to define an endophyte compartment microbiome, facilitating controlled dissection of plant-microbe interactions derived from complex soil communities.

# contributed equally to this work.

## S3.1

16:00-16:30

Jeffery L. Dangl<sup>1,2,9,10,11</sup>,  
Derek S. Lundberg<sup>1,2,#</sup>,  
Sarah L. Lebeis<sup>1,#</sup>, Sur  
Herrera Paredes<sup>1,#</sup>,  
Scott Yourstone<sup>1,3,#</sup>,  
Jase Gehring<sup>1</sup>,  
Stephanie Malfatti<sup>4</sup>,  
Julien Tremblay<sup>4</sup>, Anna  
Engelbrektsen<sup>4</sup>, Victor  
Kunin<sup>4</sup>, Tijana Glavina  
del Rio<sup>4</sup>, Robert Edgar<sup>5</sup>,  
Thilo Eickhorst<sup>6</sup>, Ruth E.  
Ley<sup>7</sup>, Philip Hugenholtz<sup>4,8</sup>  
and Susannah Green  
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## Identifying bacterial traits mediating epiphytic fitness

## S3.2

16:45-17:15

Steven Lindow

Transcriptome analysis of cells of *Pseudomonas syringae* recovered from leaf surfaces reveal that genes for flagellar motility and chemotaxis as well as phosphate uptake are prominent among the many plant-induced genes, suggesting that active exploration of the leaf, presumably to access localized sites of nutrient abundance facilitates colonization. The production of 3-indole acetic acid, a trait found in a large percentage of epiphytic bacteria, appears to activate plant cell wall invertases that results in a conversion of sucrose to fructose and glucose, both of which can be accessed at lower concentrations by bacteria than sucrose itself. The composition of the epiphytic bacterial communities on plants might be influenced not only by the plant itself, but also by fungal endophytes and epiphytes. *Neotyphodium* sp., intercellular fungal colonists of cool season grasses, produce loline alkaloids that can be consumed by a specialized group of bacteria. The lolines appear to constitute the major source of Carbon and Nitrogen available to bacteria on fungal endophyte-infected grasses, and a majority of bacteria recovered from such plants catabolize this resource, while such bacteria are absent from non-grasses. *P. syringae* strains can also parasitize fungi via their expression of an apoptosis-inducing protein similar to fungal HetC proteins, and achieve higher population sizes due to the nutrients released from the dead fungi. Fungi thus may thus facilitate transfer of resources from the plant to bacterial colonists.

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## Microbial life of indigenous phyllosphere bacteria

## S3.3

17:15-17:45

Julia A. Vorholt

Leaves represent one of the largest biological surfaces on Earth. Rather than existing as axenic organisms, plants are colonized by microorganisms that affect both their health and growth. To gain insight into the physiology of phyllosphere bacteria under *in situ* conditions, we performed a culture-independent analysis of the microbiota associated with leaves of soybean, clover, *Arabidopsis thaliana* as well as rice using a combined metagenomic and -proteomic approach. Protein identification allowed insights into the common and different physiological adaptation mechanisms to life in the phyllosphere. Besides, we found the predominance of few bacterial genera in the community provoking the question whether these commensals play a role as barrier against invading plant pathogens. To test this, an *in planta* assay was applied with *A. thaliana* as the model plant and *Pseudomonas syringae* pv. *tomato* DC3000 as the model pathogen. *Sphingomonas* strains were tested as potential antagonist under gnotobiotic conditions and showed a plant-protective effect by diminishing pathogen growth and suppressing disease symptoms. Possible mechanisms underlying plant protection will be discussed as well as the adaptation of these bacteria to life on leaf surfaces.

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## Session 4: Genetic control and selection of plant microbiomes

*Chair: Julia Vorholt*

### Plant-bacteria interaction in the rhizosphere based on signalling compounds related to bacterial quorum sensing

# S4.1

09:00-09:30

**Anton Hartmann**

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Quorum sensing (QS) is a behaviour of bacteria which is mediated by small diffusible autoinducer molecules. In Gram-negative bacteria, N-acylhomoserine lactones (AHL) are the most frequent quorum sensing molecules. In addition, the quality of the cell environment is reflected in the concentration of the signal molecules and thus the QS-compounds also sense the habitat quality in a kind of 'efficiency sensing'. In this way, pathogenic as well as beneficial and symbiotic bacteria regulate gene expression in an optimized manner.

A variety of plants excrete mimic compounds and/or so-called quorum quenching activities to interfere with this bacterial signalling which could result in the stimulation of virulence factors of pathogenic bacteria. In recent years, it has been found, that bacterial quorum sensing compounds are specifically perceived by plants (as well as other eukaryotic organisms), with are colonized by QS-active bacteria. Depending on the structure of the signalling molecule and the plant, AHL-compounds can induce systemic resistance or they alter the hormonal balance, leading to altered growth behaviour. Examples of different responses of Arabidopsis to different AHL-structures (with long or short side chain) will be presented as well as details of the signal perception and transport of AHL-compounds in plants.

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## Genetic control of maize, *Sarracenia*, *Setaria* and switchgrass microbiomes

# S4.2

09:30-10:00

**Jeff Bennetzen**

All plants exist in environments that are replete with a vast diversity of microbial species and all provide unique niches for microbial colonization and exploitation. In return, many of these microbes provide specific advantages to the host, such as improved mineral uptake or protection against pathogens, pests and parasites. However, in only a few cases are we beginning to understand the biological basis of the co-evolved relationships between plants and their microbial commensals or mutualists. My laboratory, in collaboration with the Baucom (Univ. Cincinnati), Devos (Univ. Georgia) and Malmborg (Univ. Georgia) labs, is investigating the use of metagenomic data as a phenotypic score for segregating plant genetic determinants of microbial populations in the rhizosphere and other plant-associated domains. Our preliminary data have shown dramatic differences in rhizosphere microbial composition derived from a single gene difference between otherwise isogenic maize lines. Our experiments with two pitcher plant species in the genus *Sarracenia* (and their intercross progeny) have uncovered huge environmental differences in some pitcher components, but not others, and have found that some species-specific differences should be available to genetic mapping in the current F2 population. Finally, analyses of segregating microbial characteristic are underway in the biofuel feedstock switchgrass and in the model C4 grass, *Setaria*, with promising early results.

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## Organization and control of the rice microbiome

## S4.3

10:00-10:30

Studies on the beneficial effects of plant-microbial associations have generally been limited to specific microbial species in different plant models. Microbes have been shown to aid plants in the absorption and assimilation of important nutrients and have been shown to protect plants from pathogenic bacteria. But when considering the plant microbiome in its entirety, we need to understand how plants affect their microbial environment, and conversely, how the plant responds to the presence of these complex communities of microbes. To address these questions, we are growing rice plants hydroponically, and using the addition of soil isolates from field grown rice (*Oryza sativa*) to analyze the transcriptional response to the microbiome. We have also begun a characterization of the microbiome of field grown rice by 16S rRNA profiling. We find significant differences between bacterial communities closely associated with rice roots and free-living bacterial communities in the soil, with the composition of the latter exhibiting a distance-dependent relationship. These results are consistent with models of selective microbial recruitment.

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## Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota

## S4.4

11:15-11:45

We have employed sequence-based bacterial 16S rRNA ribotyping to define the *Arabidopsis thaliana* root microbiota. We show that roots of *A. thaliana*, grown in different natural soils under controlled environmental conditions, are preferentially colonized by Proteobacteria, Bacteroidetes, Chloroflexi and Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. The identification of soil type-specific members within the root-inhabiting assemblies supports our conclusion that these represent soil-derived root endophytes. Surprisingly, plant cell wall features of other tested plant species appear to provide a sufficient cue for the assembly of ~30% of the *Arabidopsis* bacterial root-inhabiting microbiota, with a bias for Betaproteobacteria. Thus, this root sub-community may not be *Arabidopsis*-specific but saprophytic bacteria that would naturally be found on any plant root or plant debris in the tested soils. In contrast, colonization of *Arabidopsis* roots by members of the Actinobacteria depends on additional cues from metabolically active host cells.

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## Dissecting a ‘coastal marine microbiome’: Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom

Phytoplankton blooms characterize temperate ocean margin zones in spring. We investigated the bacterioplankton response to a massive diatom bloom in the German Bight of the North Sea by comparative 16S rRNA sequence analysis, fluorescence in situ hybridization, metagenomics and metaproteomics and observed a dynamic succession of flavobacterial and gammaproteobacterial populations at genus level resolution.

Taxonomically distinct expressions of carbohydrate-active enzymes, transporters, in particular TonB-dependent transporters and phosphate acquisition strategies were found, indicating that distinct populations of *Bacteroidetes*, *Gammaproteobacteria* and *Alphaproteobacteria* acted like guilds specialized for successive algal-derived organic matter decomposition. Our results suggest that algal substrate availability provided a series of ecological niches in which specialized populations could bloom. This reveals how planktonic bacterial species, despite their presumably homogenous habitat, can evade extinction by direct competition. Despite the great potential of cultivation-independent investigations of complex microbiomes and in situ methods, future studies must include the enrichment, cultivation and detailed physiological investigation of relevant strains of *Bacteria* and *Archaea* to test our hypotheses.

## Keynote Plenary 2

18:30-19:30

**Rudolf Amann**  
*et al.*

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## Session 5: Characterization and ecology of plant microbiota

*Chair: Jan Dirk van Elsas*

### Beneficial interactions of endophytic bacteria on their host plants

# S5.1

09:00-09:30

**Daniel van der Lelie and Safiyh Taghavi**

Plants live in close association with microorganisms, including rhizospheric, endophytic and epiphytic bacteria and fungi. To better understand the interactions between poplar and its beneficial endophytic bacteria, the genomes of four endophytes were sequenced: *Enterobacter* sp. 638, *Pseudomonas putida* W619, *Stenotrophomonas maltophilia* R551-3 and *Serratia proteamaculans* 568. Comparative genomics between endophytic bacteria and closely related species revealed niche specific adaptation for microbe-host interactions. Often, these adaptations are encoded on genomic islands or on plasmids. For instance, the plant growth promoting endophytic bacterium *Enterobacter* sp. 638 can improve the growth of poplar on marginal soils by as much as 50%. Analysis of its genome sequence, combined with metabolite analysis and quantitative PCR pointed to a remarkable interaction between *Enterobacter* sp. 638 and its poplar host with the endophyte responsible of the production of several phytohormones, and a precursor for another that poplar is unable to synthesize, and where the production of the plant phytohormones depended on the presence of plant synthesized compounds, such as sucrose. Whole transcriptome analysis was used to further understand the adaptation of *Enterobacter* sp. 638 and its host, and provided insights in how the in planta cell density of this endophyte was regulated.

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## Micro-managing sustainability: Ecology of the *Miscanthus*-associated microbiome

## S5.2

09:30-10:00

Angela D. Kent

Sustainability is a key economic and environmental issue in agricultural systems, but it is particularly critical for bioenergy feedstock production. Basic questions about the ecology of plant-associated microbes need to be answered if these plant-microbe associations are to be managed to enhance sustainable and economically viable production of biofuel feedstocks, such as *Miscanthus*, a C4 perennial grass that is a candidate biofuel feedstock in Europe and the US. Using DNA sequencing and community fingerprinting approaches, we describe *Miscanthus*-associated microbial assemblages in native, agronomic, and naturalized populations of *Miscanthus*, and we examine an array of biotic and abiotic factors with the potential to influence the community composition of endophytic and rhizosphere bacteria associated with *Miscanthus*. Niche (endophyte or rhizosphere) and plant genotype play an important role in shaping the microbiome. Edaphic factors are strong ecological drivers, particularly for rhizosphere microbial assemblages. DNA sequencing indicates that Proteobacteria dominate the *Miscanthus* microbiome, particularly Betaproteobacteria genera such as *Burkholderia*. A number of nitrogen-fixing genera were also detected, suggesting that this functional group may contribute to *Miscanthus* sustainability. Evaluation of key ecological drivers that shape *Miscanthus*-associated microbial populations will identify environmental factors that are critical to monitor in order to optimize beneficial plant-microbe interactions.

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## Factors shaping the microbial community within *A. thaliana*

## S5.3

10:00-10:30

**Joy Bergelson**

Host-pathogen interactions are often viewed, and studied, within the context of a closely coupled interaction. However, not only do hosts harbor a wide diversity of microbial species, these microbial species generally associate with multiple hosts. There is mounting evidence that community complexity has profound implications for the ecological and evolutionary interactions between hosts and their associated microbes. I will describe both culture based and non-culture based characterizations of the microbial community associated with *Arabidopsis thaliana*, and discuss some recent illustrations of how this ecological complexity shapes the evolution of species interactions. As an alternative to dissecting two-species interactions, we have initiated two parallel efforts to understand how *A. thaliana* hosts shape the microbial communities that reside within them. First, we are asking how well one can predict microbial community structure based on measures of species interactions. Second, we are seeking to identify host factors that have broad reaching impacts on microbial community structure. I will provide a progress report on these efforts.

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## Culture-independent community profiling and directed isolation of the sugarcane rhizosphere

## S5.4

11:15-11:45

Sugarcane is one of Australia's primary crops worth 1.75 billion dollars annually. High sugar yields have been achieved with breeding high-yielding and pest-resistant elite varieties in combination with agronomic measures that include high fertiliser application rates. Despite a high-input agronomy, there is much concern that sugarcane yields are declining and pollution footprints increasing. The main reason for this decline is considered to be deteriorating soils including soil biological properties. While the importance of soil microbial communities for plant health and nutrient cycles is widely appreciated, limitations imposed by traditional culture-based microbiology have hindered progress.

Here, we used modern molecular techniques to obtain the first culture-independent snapshot of the sugarcane rhizosphere. Specifically, we amplified the small subunit ribosomal RNA (SSU rRNA) gene from bulk DNAs extracted from the roots and surrounding soil of widely-grown commercial Queensland cane variety Q208 using barcoded primers broadly targeting bacteria and archaea (803F and 1392R). Pooled amplicons were pyrosequenced and analysed using a QIIME-based pipeline. We analysed 3 biological replicates of plants grown in low (20 kg/ha) and high (140 kg/ha) N plots in tropical Australia. Rhizosphere communities were similar in low and high N-fertilised soils after a pronounced wet season in late summer. There were however striking differences between bulk soil and rhizosphere community profiles including a 22-fold increase in a *Burkholderia* phylotype and >5-fold enrichment of a number of other genera including phylotypes belonging to *Streptomyces* (19-fold), *Bacillus* (17-fold), *Micrococcus* (12-fold), *Terrabacter* (8-fold), *Actinoplanes* (6-fold), *Duganella* (6-fold), *Blastococcus* (6-fold) and *Rhizobium* (6-fold).

Since many of the enriched sugarcane rhizosphere populations have closely related cultivated relatives, we obtained representative cultures using a directed isolation approach. Axenic cultures of *Bacillus*, *Rhizobium* and *Burkholderia* were identified by multiplex-SSU rRNA PCR which were subsequently found to have 100% sequence identity to the dominant *Bacillus* (20% of rhizosphere community) and a flanking *Rhizobium* (1.3%) and *Burkholderia* (0.77%) phylotype respectively. All three isolates were capable of growth on N-free medium suggesting that they can fix N<sub>2</sub> and all three isolates stimulated root growth of Q208 plantlets in axenic culture. We are exploring the nature of this interaction using comparative metatranscriptomics of different host-microbe pairings. Our project will integrate knowledge of rhizosphere microbial communities from field-grown sugarcane to plant performance in controlled systems. Such knowledge will provide avenues for targeted management of soil biology for sustainable sugarcane production.

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## Plant microbe interactions in the *Populus* rhizosphere

## S5.5

11:45-12:15

Diversity and complexity metrics of the microbiome associated with long lived perennial plants are among the highest of any surveyed host microbial system. The genus *Populus* contains some of the oldest genets among all plant species and thus potentially provides a stable ecological niche for development of a rich microbial community. We have sampled and phylotyped rhizospheric and endophytic communities associated with 25 *Populus deltoides* genotypes representing two geographically separated river drainages in each of two years from both spring and fall collections in each year. A total of 62 individual field collections were made in 2010 and 2011. In addition to phylotyping we were able to isolate ca. 1200 bacterial and several hundred fungal strains from these collections. The microbial isolates have been screened using a combination of microbial functional assays, host phenotypic screens, and host reporter genes using marker gene expression from selected metabolic pathways. Antagonistic and putative mutualistic isolates have been identified using re-colonization experiments. We sequenced, assembled and annotated the genomes of 43 of these isolates. Metabolic profiling has been used to identify chemical signals that may play a role in host microbe interactions. Potential mycorrhiza helper bacteria have been identified that favorably influence the fungal colonization of the *Populus* host.

**G. Tuskan, C. Schadt, D. Pelletier, J. Labbe, D. Weton, T-Y. Lu, P. Lankford, T. Tschaplinski, N. Engel, S. Jawdy, L. Gunter, M. Doktycz**

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# Poster Abstracts

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\* S=speaker abstract; P=poster abstract

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## Microbial fingerprints in the rhizosphere

P1

**KRISTIN ALEKLETT, MIRANDA HART**

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One of the ultimate goals in ecology is to understand how communities are formed. Though patterns in plant and animal communities are becoming more evident, it is not yet clear what factors shape microbial communities in plants. Plants live surrounded by, and in cooperation with microbes. The close relation between plants and microbes has shown to affect plant productivity, stress tolerance and even reproduction. We know both abiotic and biotic factors affect microbes, but we do not fully understand how a plant acquires its microbial community, how much these communities vary among individual plants or to what degree individual microbial communities determine plant fitness. Our research examines individual rhizosphere communities from a population of *Pilosella aurantiaca* (L.) F.W.Schultz & Schultz-Bip (Orange hawkweed) known to have extremely low genetic diversity in North America. This is an ideal system in which to tease apart environmental factors from genetic. Our goal is to identify the relative importance of biotic and abiotic factors in shaping distinct microbial communities among plant rhizospheres.

## Characterization of native root fungal endophytes and their impact on tomato plant growth and health

P2

**D. R. ANDRADE-LINARES<sup>1</sup>, K. H. REXER<sup>2</sup>, G. KOST<sup>2</sup>, S. RESTREPO<sup>3</sup>, R. GROSCH<sup>1</sup>, M. C. CEPERO<sup>3</sup>, E. MAXIMOVA<sup>4</sup>, A. KRUMBEIN<sup>1</sup> P. FRANKEN<sup>1</sup>**

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Isolation of native microorganisms being intimately associated with roots and characterization of their impact on plant performance is a prerequisite for understanding the so-called rhizosphere effect in modern plant production systems. This study was aimed to identify native endophytic fungi from roots, to evaluate their effect on plant development and resistance and their influence on plant product quality. Tomato (*Solanum lycopersicum*) was chosen as model because it is an important fresh market vegetable and frequently used for genetics and molecular physiological studies. Root samples of healthy plants were collected and disinfected fragments were incubated on agar media. Fungal isolates were characterised by morphological and molecular features. Root colonization was analysed *in vitro* by confocal microscopy and impact on plant growth and development was assessed. A total of fifty one fungi were obtained after root surface sterilization and 14 isolates were characterised up to species level showing their taxonomic position among the Ascomycota, including new members of dark septate endophytes (DSE). Experiments under greenhouse conditions showed differential influence of the 14 isolates on various plant growth parameters. The impact on plant health and yield of three DSE isolates (E48, E49 and *Leptodontidium orchidicola* strain E135) were evaluated in more detail. *L. orchidicola* increased biomass and glucose content of tomato fruits at early date of harvest while two DSE (E49 and *L. orchidicola*) decreased the negative effect of *Verticillium dahliae* at low pathogen dosage. This study established a basis for further investigations of the interaction between DSE and vegetable plants.

## Characterization of the root mycobiome in the perennial Brassicaceae species *Arabis alpina*

P3

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Plant species of the *Brassicaceae* have lost the ability to form the mutualistic symbiosis with arbuscular mycorrhizal fungi which normally provides host plants with essential nutrients in exchange for plant-based carbohydrates. The goal of this study is to characterize the endophytic fungal microflora of the perennial *Brassicaceae* species *Arabis alpina* and to identify fungal root endophytes which share a functional role which is similar to that of mycorrhizal fungi. Endophytes are microbes that live within plant tissues without causing disease symptoms. However, an endophyte may not remain asymptomatic throughout its life cycle which implies that endophytic species may have one or multiple functional roles, parasitic, commensalistic or mutualistic, during their life cycle or in response to plant or environmental cues. Interactions with beneficial endophytic microbes can be exploited to enhance plant performance. *Arabis alpina* plants were isolated from two different geographical sites. An initial survey of fungal species diversity was performed by PCR phylotyping using ribosomal DNA from washed roots and of fungi isolated from these roots and grown in culture. BLAST searches revealed >50 operational taxonomic units the vast majority of which could be assigned to Ascomycetes. The functional role of two culturable non-pathogenic fungi described as root endophyte or root associated fungus, respectively, is currently being investigated in association with *A. alpina* and *Arabidopsis thaliana* under different environmental conditions.

This study has been performed prior to planned comprehensive diversity studies using next-generation-sequencing. A challenge is to understand the ecological significance of phylotypic diversity.

## Reconstruction of root associated bacteria community in *Arabidopsis*

P4

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Little is known about the structure and function of plant Root-Associated Bacteria (RAB). Recent study shows that *Arabidopsis* roots in nature soil are preferentially colonized by Proteobacteria, Bacteroidetes, Chloroflexi and Actinobacteria. To further study the role of RAB, we try to isolate bacteria from *Arabidopsis* root from nature soil and sequence their genomes by next generation sequencing techniques.

## ***Pseudomonas fluorescens* F113 produces a second flagellar apparatus which is important for rhizosphere colonization**

**P5**

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The genomic sequence of *Pseudomonas fluorescens* F113 has shown the presence of a 40 kb cluster of genes which encode the production of a second flagellar apparatus. These genes are not present in the genome of any sequenced pseudomonad and are homologous to the flagellar genes of the soil bacterium *Azotobacter vinelandii*. Regulation of these genes is mediated by the *flhDC* master operon, instead of the typical regulation through *fleQ*. Under laboratory conditions, F113 does not produce this flagellum and the *flhDC* operon is not expressed. However, ectopic expression of the *flhDC* operon is enough for its production, resulting in a hypermotile strain. This flagellum is also produced under laboratory conditions in several mutants, including the *kinB* and *algU* mutants. Genetic analysis has shown that *kinB* represses the expression of the *flhDC* operon. This operon is activated by the Vfr protein probably in a c-AMP dependent way. The strains producing this second flagellum are all hypermotile and present a tuft of polar flagella instead of the single polar flagellum produced by the wild-type strain. Phenotypic variants isolated from the rhizosphere produce this flagellum and mutation of the genes encoding it results in a defect in competitive colonization, showing its importance for rhizosphere colonization.

## **Induced systemic resistance and rhizosphere colonization by *Pseudomonas fluorescens***

**P6**

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Elicitation of induced systemic resistance (ISR) by plant-growth promoting rhizobacteria (PGPR) primes plants for enhanced defense responses upon pathogen attack. Studies on ISR by *Pseudomonas fluorescens* revealed plant determined differences between strains. Whereas *P. fluorescens* WCS417r can elicit ISR in both Arabidopsis and radish, *P. fluorescens* WCS374r is only effective on radish. Compared to WCS374r, WCS417r reaches higher populations in the Arabidopsis rhizosphere, but both strains colonize roots of radish equally well. Plants can select bacteria through the production of specific root exudates and differences in exudates between Arabidopsis and radish might explain the observed differences in colonization. In Arabidopsis, the transcription factor MYB72 was previously found to be required for ISR, and WCS417r cannot induce ISR in *myb72* mutants. This is accompanied by a strongly reduced colonization of the *myb72* rhizosphere by WCS417r. This suggests that MYB72 is involved in the plant-PGPR signaling events that lead to increased colonization by WCS417r. Mechanisms involved are studied making use of the differential effects of plant species, plant genotype and bacterial genotype on ISR and bacterial colonization.

## Uncovering the mechanisms behind unwanted *Agrobacterium tumefaciens* chromosomal DNA transfer to plants

P7

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*Agrobacterium tumefaciens* is the causative agent of crown gall disease. Its DNA transfer mechanism became the method of choice for plant transformation. Recently it was discovered that, besides genes located on the T-DNA, sometimes large *Agrobacterium* chromosomal DNA fragments (AchrDNAs) are unintentionally transferred from bacteria to plants. Although finding AchrDNA in plants has implications for our understanding of horizontal gene flow between species, it is worrisome for the use of this technology in generating transgenic plants. To develop effective solutions, it is necessary to determine the mechanism(s) behind this unwanted AchrDNA-transfer.

In a screening designed to resolve whether T-DNA gets inserted into bacterial chromosomes and re-launches from there together with flanking AchrDNA, we discovered how an *IS*-element (*IS426*) gets unintentionally transported to plant genomes. Since *IS426* was reported to be one of the 'hot spots' of AchrDNAs that frequently gets inserted into the plant genomes, we characterised its mechanism of transposition, copy number and promoter activities. This work should lead to a better understanding of the role of mobile DNA in horizontal gene-transfer, how bacteria gain new abilities like antibiotic resistance and how they adapt to environmental conditions. We discuss further possibilities how other AchrDNAs could get transferred to plants.

## Synthetic phyllosphere communities on *Arabidopsis thaliana*

P8

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Both environmental factors and genetic factors shape the plant-associated microbial community. In order to study host genetic factors, the impact of environmental factors needs to be reduced. The aim of this project is to establish a model phyllosphere microbiota in *Arabidopsis thaliana*. Seedlings were inoculated with a 7-species synthetic community, composed of strains representing the most abundant phyla in the phyllosphere. Automated Ribosomal Intergenic Spacer Analysis (ARISA) was used as a fingerprinting technique to compare communities. The model phyllosphere microbiota was stable: all 7 members are found 2 weeks after inoculation using a gnotobiotic system. Moreover, the community was reproducible: communities from two independent experiments could not be distinguished and variation between samples was found to be low. By contrast, the ARISA method was able to discriminate microbiota associated with plants grown under different environmental conditions, i.e. using an open system with lower humidity. Next, a screen of *A. thaliana* mutants will be performed to identify *a priori* candidate genes with potential impact on the colonization of the phyllosphere.

## Deciphering the root-associated bacterial microbiota of cultivated barley

P9

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Dissecting the structure and the metabolic potential of the plant microbiota represents a key step towards utilization of these microbes for crop improvement. We have explored the barley root-inhabiting microbiota through a controlled environment experiment in which a cultivated barley variety was grown in natural soil and compared with the communities retrieved from the root of the model plant *Arabidopsis thaliana*. We used the barley variety Keel, whose flowering time is comparable with the one of *A. thaliana* in the tested condition, and we performed a 16S rRNA gene pyrosequencing profiling of the bacterial root-inhabiting microbiota. Consistent with previous observations (Bulgarelli *et al.*, in revision) the two species share a large proportion of microbes enriched from the unplanted soil. Remarkably, a subset of bacterial ribotypes significantly discriminates the barley communities from the one retrieved from the root of *A. thaliana* and the unplanted soil. Interestingly end-point PCR assay with a marker designed on the bacterial *amoA* gene revealed a specific enrichment of nitrifying bacteria in the communities associated to barley roots. These results indicate that both bacteria phylogenetic and functional markers represent a tool to further dissect the genetic relationship between barley and its root-associated microbial communities.

## Root induced modulation of microbe composition by rice plants

P10

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Plant roots are known to excrete up to 10 to 40% of their photosynthate as root exudate into the surrounding soil, and it is known that this process is important for improving the bioavailability of nutrients such as phosphorus, but root exudates have also been implicated in the recruitment of soil microbes. Some rhizosphere-associated microbes are known to provide various services to plants such as nitrogen fixation and disease suppression. The establishment of a structured microbe community around plant roots may be an important step in achieving and maintaining a plant rhizosphere that can maximize nutrient accessibility and resist plant pathogens. The degree to which plants are able to affect the taxonomic structure of their rhizospheric microbe population is not yet known. Here we report a preliminary analysis of the local taxonomic structure of microbe communities associated with soil grown rice plants. Differences in the microbial communities between the root soil interface and more distant sites within the rhizosphere were detected. Root associated samples could be separated from more distant samples using principal coordinate analysis, and the OTU composition between these was found to be statistically significant using permutational MANOVA. Indicator species analysis revealed approximately 10 OTUs that were more closely associated with the rice roots.

## Characterisation of microbial endophytes from wild grapevine (*Vitis vinifera* subsp. *sylvestris*)

P11

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While a large number of studies aim at analyzing the microorganisms found in crops, using either a classical microbiology approach or more novel, DNA-based techniques, little is known on the selective forces affecting these microbial communities during the process of domestication or if and how they shaped plant-associated microbes. We are interested in elucidating how domestication has influenced bacteria and fungi colonizing the grapevine endosphere, and how these modifications interfere with plant physiology, growth and health.

We isolated microbial endophytes from *V. vinifera* subsp. *sylvestris* plants obtained from different areas of Italy and analysed the isolates for important traits related to interaction with the plant host, social behaviour, tolerance to antibiotics and their production, biocontrol.

In addition, a DNA-dependent approach was adopted to fingerprint microbial communities in wild grapevine plants and to compare them to non-domesticated plant. Automated Ribosomal Intergenic Spacer Analysis (ARISA) was used to assess variability and identity of the non culturable microbial fauna. Both geographical origin and plant genotype were considered.

The ability of some of these isolates to colonize domesticated grapevines was investigated to assess their viability for reintroduction in cultivated grapevines.

## Diversity of endophytic bacterial communities in *Pinus flexilis* foliage

P12

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The relationship between forest trees and the endophytic bacteria is likely to be one of the world's most wide-spread symbioses, yet, we presently have limited knowledge of bacterial diversity and function within forest trees. Using 454 pyrosequencing with primers targeting bacterial 16S rRNA genes, we explored the diversity of foliar endophytic bacteria associated with mature *Pinus flexilis* in Niwot Ridge, CO. The endophytic community from 6 individuals of *P. flexilis* sampled at two sites were all dominated by *Alphaproteobacteria* (50-70%), with little variation in bacterial richness and OTU abundance among and within individuals. The dominant genera were an unclassified Acetobacteraceae, *Gluconacetobacter* sp., and *Acetobacter* sp., together making up 45-60% of total bacteria in the sample. These genera have been identified as nitrogen fixers in other plants. The presence of endophytic nitrogen fixers was confirmed with PCR amplification of the nitrogenase reductase gene, *nifH* in each sample. This suggests that *P. flexilis* endophytic communities are dominated by a core community of nitrogen-fixing endophytes. Thus, although commonly attributed to soil bacteria, nitrogen-fixation in forests could also be carried out by endophytes.

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Jasmonates (JA) are plant hormones involved in both deleterious and beneficial plant-microbe interactions. In response to herbivorous insects and necrotrophic pathogens, plants activate the JA signalling pathway. This activation triggers a cascade of events that culminate in physical and chemical barriers that combat the attack. Jasmonates also regulate 'priming' by beneficial soil microbes in response to pathogen attack, a phenomenon known as Induced Systemic Resistance (ISR). It is largely unknown how modulation of the plant JA signalling pathway affects the diversity of the rhizosphere microbiome. In this study we used 16S rRNA gene amplicon pyrosequencing to characterize the rhizosphere microbiome collected from JA-treated wild-type *Arabidopsis thaliana* and the mutants *jin1/myc2* and *pft1*, which are impaired in the JA signalling pathway. When compared to the control plants, the composition of microbial communities was significantly altered in the rhizosphere of JA-treated and JA signalling mutant plants. Enriched microbial populations potentially involved in beneficial and deleterious plant microbe interactions were identified. Conspicuously, known groups of beneficial microbes were significantly enriched during jasmonate signalling. Overall, our study demonstrates that plants could modulate the rhizosphere microbiome when activating the JA signalling pathway.

## Metatranscriptomics of the rhizosphere microbiome; the quest for bacterial genera and traits involved in natural plant protection

P14

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In natural disease suppressive soils, plants are protected from fungal infections in spite of the presence of the pathogen. Disease suppressiveness is, in many cases, microbial in origin. For various fungal pathogens, soil suppressiveness develops in the field after several years of high disease incidence. Hence, the fungal pathogen appears to be required for activating specific antagonistic microorganisms. PhyloChip-based metagenomics of a soil suppressive to *Rhizoctonia* damping-off disease uncovered the bacterial diversity of the rhizosphere microbiome. To get insight into the active microorganisms and traits expressed during disease suppression, rhizospheric RNA from sugar beet plants growing in suppressive soil with or without the fungal pathogen was sequenced. Analyses of over 5 million sequencing reads revealed more than 1,000 bacterial taxa. The overall structure of the bacterial community was in accordance with the previous metagenomic results, with Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Planctomycetes as the dominant phyla. Bacterial taxa that were the most enriched in presence of the fungal pathogen belonged to the  $\alpha$ - and  $\beta$ -Proteobacteria, Actinomycetales and Sphingobacteria. mRNA sequence analyses revealed more than 200,000 proteic features representing different subsystems categories, including motility, chemotaxis, membrane transport and secondary metabolism. Results of the taxon and function-targeted analyses will be presented.

## Accessing the diversity of bacterial endophytes in the energy crop *Miscanthus*

P15

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*Miscanthus* is a perennial grass which is currently being developed as an energy crop to substitute fossil fuels. It combines the efficiency of C4 photosynthesis with a tolerance to temperate climates and is therefore capable of producing high biomass yields across a wide geographical area whilst having little or no requirement for inputs such as nitrogen fertiliser.

Endophytes are microbes which live within the tissues of a plant without causing visible detriment to the host. Bacterial endophytes have been demonstrated to confer significant advantages to sugarcane, a close relative of *Miscanthus*, in terms of nitrogen fixation; further benefits have been reported in other plant species, including increased biomass and tolerance to biotic and abiotic stresses.

We here describe a molecular-based analysis of the diversity of bacterial endophytes present within *Miscanthus*. Different populations comprising multiple phyla were identified in leaves, stem, rhizome and roots, with a general similarity within above ground tissues and below ground tissues, but with fewer similarities between these two groups. Studies to determine any benefits to the plant host which may be exploited for crop improvement, including putative nitrogen fixation, are ongoing.

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## Phylloplane yeast associated with strawberry

P16

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Phylloplane yeast populations on strawberry leaves and fruits were assessed via plating and denaturing gradient gel electrophoresis (DGGE). The dominant yeast species detected from DGGE were minor or non-growers on the plates, whereas only one yeast isolate detected on the culture plates could be attributed to a represented strain in DGGE. Both on plates and in DGGE, the dominant yeasts belonged to the genera *Cryptococcus*, *Rhodospiridium*, and *Sporidiobulus*. Examination of the dynamics of the phylloplane yeast populations revealed differences in diversity and density depending on the growing system, strawberry tissue and sampling time. In general, immature fruits showed significantly larger populations than mature fruits or leaves.

We also studied the effect of the applications of two commonly used fungicides on the yeasts. Using both plating and DGGE, we observed only a minor impact of the fungicides on the compositions and concentrations of the phylloplane yeast communities. These results demonstrate the existence of fungicide resistance among phylloplane yeasts of strawberry and suggest the potential to use these yeasts in integrated control of important fungal diseases of strawberry.

## Endophytic microbiome of rice: A significant outlook towards agriculture in Indian region of Indo-Burma Biodiversity Hot spot

P17

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With the alarming food crisis scenario worldwide, there is continuous exploration of microbes associated with plant crops to meet the ever increasing demand. Keeping it as one of the important criteria, indigenous local rice variety of Manipur from different geographical locations was chosen as the target host for exploring potential endophytic microbiome. This study aim to characterize the microbial communities inhabiting rice root, culm & leaves to identify deterministic factors influencing plant growth and biocontrol activity against widely prevailing pathogens of rice especially *Pythium sp.* and *Sclerotium oryzae* that affects the root, which is a vital part for all functions. Healthy plants were chosen for sampling with effective sterilization strategy. Some of the dominant endophytes associated are *Acremonium sp.*, *Trichoderma sp.*, *Aspergillus sp.*, *Cyrtaria sp.*, *Penicillium sp.*, *Epicoccum sp.*, *Colletotricum sp.*, *Gibberella sp.*, *Fusarium sp.*, *Cochliobolus sp.*, *Phoma sp.* *Enterobacter*, *Pseudomonas sp.*, *Bacillus sp.* including pigmented bacteria and yeast. Mechanisms such as protease, chitinase, siderophore, P-solubilization, IAA activity, growth assessment on nitrogen free medium were performed. Among the isolates; Mz10, T-O, D108A (bacteria), and D56A & D34F (fungus) showed promising activity and mode of action of these endophytic isolates were examined for possible utilization in sustainable agriculture in NE India.

## Diversity and abundance of N-cycling bacteria associated with nitrous oxide emissions in a corn-based biofuel production system

P18

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Opportunities exist in agriculture to use corn residues (*Zea mays* L.) for biofuel production; however, the consequence of removing residue on soil microbial community activity is unknown. Nitrous oxide (N<sub>2</sub>O) is the most important greenhouse gas (GHG) emitted from agroecosystems and is a byproduct of microbial nitrification and denitrification. Using micrometeorological approaches to measure N<sub>2</sub>O at our field site, it was determined that conventional tillage and removal of crop residues increased yearly N<sub>2</sub>O emissions compared to no-till systems. The majority of the annual emissions occurred during spring thaw, and were associated with alterations in bacterial community structure. Active populations of ammonia oxidizers, nitrate and nitrous oxide reducers were enumerated throughout the field season and indicated that *denovo* denitrification was occurring during a spring thaw emission event. Furthermore, the expression of *nosZ*, the gene responsible for the conversion of N<sub>2</sub>O to N<sub>2</sub> gas during denitrification was inversely related to N<sub>2</sub>O emissions. Suggesting that at our field site differences in the magnitude of N<sub>2</sub>O released during spring thaw may be due to soil conditions that support incomplete denitrification. This is the first work to relate changes in abundance of active of nitrifiers and denitrifiers to year round field-scale measurements of N<sub>2</sub>O.

## The core and pan-genome of serpentine-adapted *Mesorhizobia*

P19

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Microbial associates may enable plants to express adaptive functional traits. Rhizobia can alter their legume hosts' abiotic stress responses; furthermore, rhizobia have variable stress tolerance. Here, we characterize the draft genomes and nickel tolerance of a collection of 48 *Mesorhizobium* isolates from paired serpentine and non-serpentine soils across California. We identify genes iteratively using the *Mesorhizobium loti* full genome sequence and Genbank. Strain relationships are similar whether based on reference gene content, non-reference gene content, or sequence similarity; this indicates most genes are not horizontally transferred. We find significant but weak population structuring by soil type and geographic location (average genome-wide F[soil/total] = 0.030 and F[location/total] = 0.062). Strains from serpentine sites show higher growth in the presence of nickel; furthermore, within a focal subclade of 38 isolates the number of genes identified is positively related to growth rates both with and without nickel. Association mapping reveals multiple genes whose presence/absence correlates with growth in nickel, including genes involved in conjugal transfer and pili formation. Our study highlights the accessory genome's involvement in rhizobium serpentine adaptation and provides a foundation for understanding how these strains impact local adaptation of their hosts.

## Microbial communities associated with the bio-energy plant *Miscanthus*

P20

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Endophytic bacteria have enormous potential as biofertilisers, biocontrol agents and in improving the phytoremediation capacity of plants. This study investigated the culturable-aerobic bacterial diversity associated with the bio-energy plant *Miscanthus giganteus*. 250 bacterial strains were isolated from *Miscanthus* and 70 of these strains were identified through 16S rDNA sequencing. Eleven different species were identified in *Miscanthus* originating from the leaf, stem and rhizome tissues. The majority of these isolates were *gamma-Proteobacteria* with *Pseudomonas* and *Acinetobacter* species dominating. Many of these strains expressed plant growth promotion traits such as phytohormone production and phosphate solubilisation ability. The majority of the isolates were found to possess resistance to heavy metals and 7-13% possessed inherent organic xenobiotic degradation abilities. A number of these isolates were tagged with a *gfp*:kanamycin marker and were found to colonise the rhizosphere of inoculated plants. These isolates may prove to be useful inoculants for improving plant biomass and phytoremediation efficiency of *Miscanthus*.

## Endophytic bacterial communities of switchgrass (*Panicum virgatum* L.) in the native tallgrass prairie of Oklahoma

P21

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Switchgrass plant samples were collected from the tallgrass prairie of northern Oklahoma in spring, summer and fall of 2009. Endophytic bacteria were isolated in pure culture from roots, and the 16S rDNA region was sequenced to provide a taxonomic identification. Bacteria were grouped into communities based on phylum, class and genus, as well as collection season. The Shannon diversity index was used to estimate species diversity and the diversity indices were compared using a t-test. The sequences analysis of 453 bacterial strains revealed that they belonging to five classes, 17 families and 29 genera. The species diversity was the highest in spring and was the least in summer. All three temporally distinct populations were significantly different in species diversity ( $P < 0.02$ ). Two bacterial strains from this study, *Pseudomonas* spp. and *Enterobacter* spp. representing most abundant and relatively less abundant genera, respectively, were tested in the greenhouse on a switchgrass cv. Alamo for biomass production. Inoculation of either bacterial strain increased plant height, above ground biomass and below ground biomass. Our data suggest that natural populations of switchgrass harbor diverse groups of endophytic bacteria with the potential for agricultural application.

## Crosstalk between PAMP-triggered immunity and photosynthesis

P22

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The innate immune system allows plants to respond to potential pathogens in an appropriate manner while minimizing damage and energy costs. Photosynthesis provides a sustained energy supply and therefore has to be integrated into the defence against pathogens. Although changes in photosynthetic activity during infection have been described, a detailed and conclusive characterization is lacking. Here, we addressed whether activation of early defence responses by pathogen-associated molecular patterns (PAMPs) triggers changes in photosynthesis. We show by proteomics and chlorophyll fluorescence measurements that activation of defence by PAMPs leads to a rapid decrease in non-photochemical quenching (NPQ). Conversely, NPQ also influences several responses of PAMP-triggered immunity. In a mutant impaired in NPQ, apoplastic ROS production is enhanced and defence gene expression is differentially affected. While induction of the early defence markers *WRKY22* and *WRKY29* is enhanced, induction of the late markers *PR1* and *PR5* is completely abolished. We propose that regulation of NPQ is an intrinsic component of the plant's defence program.

## Intraspecific variation in the effect of *Aegilops triuncialis* on soil microbial communities, competitive ability and invasion dynamics

P23

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Plant-induced changes on the rhizosphere is a rich area of research, but few have considered intraspecific variation in these types of plant-soil interactions. I am taking such an evolutionary approach in *Aegilops triuncialis*, a cleistogamous Eurasian grass invading arid and semi-arid grasslands in California. Previous studies have reported differences in soil microbial communities between invaded versus uninvaded areas, with invaded soils consequently inhibiting native forb germination. These studies sampled only a single population. Population genetic analysis has identified three invading lineages of *A. triuncialis* in California, each differing in ecological performance of the plants. We will test if the three invasive genotypes differ in alleopathic rhizosphere effects, as well as variation in competitive ability. These studies will illustrate rhizospheric impacts, the effect these changes have on the following season's native grasses, capacity for intraspecific competition, and whether there is variation in these traits among invading genotypes. The findings will inform improved management and restoration of ecosystems impacted by *A. triuncialis* invasion.

## Impact of transgenic plants expressing the quorum quenching lactonase AttM on root-associated bacterial populations

P24

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*Nicotiana tabacum* expressing the lactonase AttM (AttM) that degrades the quorum-sensing (QS) signals, and the wild type (WT) parent line were compared for their capacity to inactivate acyl-homoserine lactones (AHL) and modify the structure and QS-associated functions of bacterial community in the rhizosphere. In vitro, AttM plantlets efficiently inactivated AHL and affect the QS-regulated conjugation of *Agrobacterium*. Both of WT and AttM lines were cultivated in non-sterile soil to evaluate their impact on the rhizospheric community. Cell densities of total culturable bacteria and selected populations isolated from plant rhizospheres and rhizoplanes were comparable whatever the genotype of the plants (AttM or WT). Similarly, the percentages of culturable bacteria which naturally produce or degrade AHL signals, were identical in the compared soil samples, and independent of the plant genotype. Bacterial populations isolated from the two plant genotypes were also analyzed irrespectively of their culturability status. Analyses targeting the *rrs* gene did not reveal any significant differences within these populations. All these data indicate that bacterial population changes that could have resulted from the genetic modification of the plants are inexistent or very limited as no change linked to the plant genotype has been observed in the rhizosphere.

## Probing the influence of host genotype on rhizosphere community structure

P25

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Sequencing efforts have begun to define the structure of the plant microbiome; yet, how host genotype affects community structure is largely unknown. We have developed a high-throughput system to monitor rhizosphere colonization, in real time, using a defined microbial community and the model plant *Arabidopsis*. Seedlings are grown hydroponically in 24-well, optically clear-bottomed plates, and inoculated with microbes expressing fluorescent proteins. Bacterial growth can be quantified using a fluorescent plate reader or fluorescent microscope. In this system the pathogenic *Pseudomonas syringae* strain DC3000 grows to high levels in the rhizosphere, several strains of the plant commensal *P. fluorescens* grow to consistent intermediate levels, and most tested non-plant associated microbes, such as *E. coli*, grow poorly. Using a simple two-member community consisting of *Bacillus subtilis* (expressing GFP) and *P. fluorescens* (expressing mCherry), we are screening an *Arabidopsis* ecotype collection to identify ecotypes with differential abilities to support commensal growth. We plan to identify the plant genes responsible for any differences in rhizosphere colonization. We are also developing a more complex model microbial community composed of culturable microbes recovered from roots of *Arabidopsis* grown in greenhouse and natural soils. This low-complexity model system provides a tractable foundation to begin to dissect how host genotype affects microbial community structure.

## **<sup>13</sup>C pulse-labeling assessment of the community structure of active fungi in the rhizosphere of a potato**

**P26**

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There is growing awareness that saprotrophic fungi play an important role in rhizosphere processes. However, information is often lacking to make a proper assessment of the importance of fungi in the rhizosphere. The aim of this study was to gain a better understanding of the carbon flow in the potato rhizosphere to the saprophytic fungal community via rhizodeposition. We performed a <sup>13</sup>C pulse-labeling experiment to monitor carbon flow from potato plants into fungal communities in relation to plant genotype. The fractions of fungi receiving <sup>13</sup>C from the plant were separated from the ones not receiving carbon via RNA/PLFA-SIP. The communities of *Ascomycota*, *Basidiomycota* and *Glomeromycota* were analysed 24, 120 and 264 hours after labeling.

The community composition of active (<sup>13</sup>C labeled) and non-active (<sup>12</sup>C labeled) fungi were different for all three phyla. Ascomycetes and glomeromycetes received carbon from the plant already 24 and 120 hours after labeling while basidiomycetes were slower in accumulating the labeled carbon indicating that they are less rhizosphere competent. We conclude that both saprotrophic and mycorrhizal fungi are able to utilize C flowing into the rhizosphere quickly, and that there are large differences in the utilization of root-derived compounds within all three fungal phyla.

## **Microbial taxonomic and functional diversity associated with bioenergy *Populus* plantations of differing age and density**

**P27**

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A well recognized benefit of biomass energy from forest trees is the ability to cultivate on marginal lands not suitable for food or other agricultural crops. The high cost of nutrient additives is not economically feasible for woody biomass, so a thorough understanding of the microbial component of nutrient cycling in these plantations is key to maximizing plant growth and minimizing the effects of abiotic and biotic stresses. Long understood that microbes contribute to key ecosystem processes, the roles of microbes associated with forest trees, such as ectomycorrhizal fungi, are particularly important in the facilitation of nutrient uptake. Despite their important ecological roles, there is a paucity of information regarding taxonomic diversity and below-ground abundance associated with forest trees. We have utilized next-generation sequencing techniques (454/Roche and Illumina/Solexa) to probe seasonal soil microbial diversity in bioenergy plantations of *Populus* by setting up a two-factor completely randomized design consisting of planting density and the presence or absence of intercropping with the nitrogen fixing legume, Black Locust (*Robinia pseudoacacia*). In 2009 and 2010, both field measurements and a genomics based gene expression strategy, including below-ground microbial diversity associated with these trees were used to assess the treatment type on plant growth and accumulation of woody biomass.

## Parsley associated endophytic bacteria and chemical bases of their interactions with parsley

P28

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There is a rising interest in studying plant associated bacteria due to their prospective use in increasing plant health, yield and soil fertility, as biocontrol agents, phytoremediation, as well as their potential use in medicine. Herbal plant-microbe interactions remain mostly unexplored. While screening for plants with antimicrobial activities, we discovered that, in contrast to other tested plants, parsley carries many endophytic bacteria. To elucidate why these bacteria are especially attracted to parsley plants, we performed chemotaxis experiments using three out of eight bacterial species identified in parsley and compared them to *Agrobacterium tumefaciens* as control. Two *Pseudomonas* species tested were attracted only to the exudates from wounded parsley, basil and kalanchoe leaves. In contrast, *Bacillus* sp. was highly attracted to the exudates from non-wounded parsley leaves but not to exudates of non-wounded basil and kalanchoe leaves. Analysis of parsley-specific volatile metabolites and acetosyringone, a general wound induced volatile compound, have shown that *Pseudomonas* species were attracted only to acetosyringone, however, *Bacillus* sp. was attracted to parsley specific metabolites but not to acetosyringone. Besides further elucidating specific molecular interactions between bacteria and herbal plants, our research will have impact on the use of fresh produce like parsley in human health.

## Genomics of *Rhizobium leguminosarum* bv. *viciae* microsymbiont selection by the legume plant host

P29

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*Rhizobium leguminosarum* bv. *viciae* establishes highly specific nitrogen-fixing symbioses with different legumes (*Pisum*, *Lens*, *Lathyrus* and *Vicia*). Classic studies using trap plants suggest that different plant hosts select different rhizobial genotypes among those available in a given soil. We have applied (meta)-multigenomic approach to study this phenomenon. A well-characterized agricultural soil was used as source of rhizobia, and plants of *Pisum sativum*, *Lens culinaris*, *Vicia sativa* and *V. faba* were used as traps. Isolates from 100 nodules were pooled, and DNA from each pool was sequenced (BGI-Hong Kong; Illumina HiSeq 2000, 500 bp PE libraries, 100 bp reads, 12 Mreads). Reads were quality filtered (FastQC, Trimmomatic), mapped against reference *R. leguminosarum* genomes (Bowtie2, Samtools), and visualized (IGV). Four different reference genomes were used for each chosen ORF (16S rRNA, recA, nodA, nodD, nifH, fixN). A low level of polymorphism was observed with 16S rRNA. The remainder genes were highly polymorphic, especially within the pea-selected subpopulation, and differences among plant-selected subpopulations were clear. These results confirm previous observations regarding plant selection of specific genotypes. It is expected that further, ongoing comparative studies on differential (meta)-multigenomic sequences will shed light on the nature of plant-specific genotype selection.

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## The endophytic fungus *Fusarium solani* (strain FsK) affects host-plant physiology and has an impact on the fungal communities in the rhizosphere

P30

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The non pathogenic *Fusarium solani* strain FsK is able to colonize the roots of tomato plants conferring induced resistance against plant pathogens. The protective ability of strain Fs-K prerequisites plant responses mediated by ethylene and ABA as determined by using a genetic approach of mutant tomato plant lines. Root colonization by FsK resulted in a significant increase in plant growth in plants watered with a balanced nutrient solution compared to non-inoculated plants. Root growth was also promoted under N deficiency and stem and leaf growth under Fe deficiency. Under water deficient conditions, FsK can alleviate stress symptoms as revealed by increased photosynthesis and growth rate (increased shoot and root fresh weights) compared to non inoculated plants. Effects on biochemical pathways involved in minimizing oxidative damage under drought stress were also recorded. Furthermore, the impact of plant inoculation by FsK or a soil pathogen (FORL) on the fingerprinting-based structure of microbial communities in tomato rhizosphere was studied. The pathogen has a readily distinguished and persistent effect on the fungal community whereas FsK has a transient effect on the fungal and alpha-proteobacterial community, which is observed only during its endophytic growth. Bacteria stimulated by FsK were closely related to species that may act as biological control agents. Overall, however, the inoculation of FsK in tomato rhizosphere did not appear to have a significant impact on the diversity of non-target microbial groups inhabiting the plant rhizosphere.

## Characterization of the *Brachypodium distachyon* root-associated microbiome

P31

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Identification of plant-microbe interactions is of key agronomic importance, but so far it has been impossible to dissect the general genetic principles that govern these interactions. As a starting point, we surveyed the root-associated microbiome of the emerging model *Brachypodium distachyon* in a natural North American soil. We used the 454 platform to profile the 16s gene content of ~70 individually barcoded plant samples. These samples represent both the rhizosphere (R) and endophytic compartment (EC) bacterial communities of 5 different natural inbred accessions, across two developmental stages. We have found that the R and EC communities of *B. distachyon* roots are distinct from the bulk soil that surrounds them, and this difference is much more prominent in the EC fraction which also shows reduced diversity. Specific taxa are enriched in each fraction, and the phylogenetic distribution of these enriched taxa is consistent across the 5 accessions tested. So far we have found no statistically significant differences between the accessions in our dataset. Our results are consistent with the view that specific organisms get recruited to the plant root, and that there is limited variation of that process within a single species.

## Influence of root exudates on proteome of plant growth promoting rhizobacterium *Bacillus amyloliquefaciens* FZB42

P32

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*Bacillus amyloliquefaciens* FZB42 is a Gram-positive bacterium which possesses the ability to competitively colonize plant roots and stimulate plant growth. To elucidate the plant growth promoting processes of *Bacillus amyloliquefaciens* FZB42, a proteomic approach was used to depict secretory and cytosolic map of proteins. To simulate the growth condition in the natural environment, cells were cultured in a medium with addition of soil extract in presence or absence of root exudates in order to evaluate the role of exudates in plant-microbe interactions. Conducted results revealed high level of synergy at both cytosolic and extracellular levels in response to root exudates. Extracytoplasmic enzymes degrading plant derived residues and permeases involved in transportation of digested compounds were over-expressed in presence of root exudates. Significant over-expression of enzyme catabolising proline, YcgN, has been recorded, what makes this protein particularly interesting, since in Gram-negative rhizobacteria, genes with similar functions have been connected with establishment of interactions with host plants. Our results indicate another function of *myo*-inositol, beyond that of a nutrient source, and shed a new light on its signaling role in the symbiotic relationship. Additionally, several proteins known to induce innate plant immunity were found expressed at higher extent during transition phase.

## *Medicago truncatula* root exudates in response to *Glomus intraradices*

P33

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Arbuscular mycorrhizal fungi (AMFs) establish mutualistic interactions with roots of most terrestrial plants. The plant mechanisms used to establish a molecular dialog with AMFs is of crucial importance for targeted implementation of the symbiosis in plant production (1). A nested core collection of 32 inbred lines of an anonymous collection of 339 *Medicago truncatula* accessions (2) was employed to evaluate genetic variation in mycorrhization rate by plant cultivation on sandy soil with diverse fungal species. Four inbred lines of either outstanding strong or weak colonization levels were selected for root exudate metabolite analysis of roots in contact with fungal material of *G. intraradices*. For root exudate collection, plants are cultivated in a sterile hydroponic cultivation system. To trace differences in secondary metabolite pattern between lines in reaction to the presence of fungus, the methods of untargeted LC-MS analysis as well as SPME for volatile analysis have been chosen. First analyses revealed clear differences between exudate patterns of plant roots cultivated alone and roots subjected to fungal material. If mycorrhization efficiency can be correlated with root exudate patterns, appropriate recombinant inbred lines available for the 32 lines will be employed for identification of QTLs relevant for distinct exudate patterns.

(1) Bonfante P, Requena N (2011) Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 14, 451-457; (2) Ronfort J et al. (2006) Microsatellite diversity and broad scale geographic structure in a model legume: building a set of nested core collection for studying naturally occurring variation in *Medicago truncatula*. *BMC Plant Bio.* 6, 28.

## Community composition of target vs. non-target fungi in fungicide treated wheat

P34

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Fungicide treatments are common control strategies used to manage fungal pathogens in agricultural fields, however, effects of treatments on the composition of total fungal communities, including non-target fungi, in the phyllosphere is not well known. Yellow rust (*Puccinia striiformis*) is a common disease in wheat and within the last decade, new aggressive strains of yellow rust has caused severe epidemics that lead to substantial yield losses.

This study explored the community composition of target versus non-target fungi in yellow rust infected wheat as affected by treatment timing and dose of three fungicides. The fungal composition in bulked leaf samples and individual leaves was studied by deep amplicon 454 pyrosequencing targeting the internal transcribed spacer-1 (ITS1) region of the ribosomal DNA. Amount of yellow rust in individual samples was quantified by qPCR. Pyrosequencing resulted in 179,081 sequences from bulked leaf samples and 91,182 sequences from individual leaves excluding low quality sequences and singletons; in total 270,263 sequences clustering into 41 operational taxonomic units (OTUs). Three different treatment regimens with two of the fungicides resulted in an amount of yellow rust below detection level. Fungal diversity was stable across treatments whereas the relative abundance of individual OTUs was affected by fungicide treatment.

## The role of innate immunity in shaping the *Arabidopsis* phyllosphere microbiome

P35

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Much of our understanding of plant innate immunity stems from studies with model pathosystems. Yet despite considerable recent interest, especially in rhizosphere communities, very little is known about the naturally occurring leaf-associated microbial communities of apparently healthy plants. *Arabidopsis thaliana*, a well-characterized model angiosperm, harbors over  $10^5$  bacterial cells per square centimeter of leaf tissue when grown in an environmental growth chamber with standard potting soil as substrate. Using optimized 16S rRNA gene-targeted pyrosequencing primers, we have characterized the bacterial leaf-associated communities of various *Arabidopsis* ecotypes (from Italy, Germany, and Sweden) and defense mutants to determine a genetic basis for associated community structure. Using our system, preliminary data suggest ecotype-specific community clustering and amplification of specific operational taxonomic units (OTUs) upon manipulation of salicylic acid signaling. Furthermore, our data suggest that microbe-associated molecular pattern-triggered immunity (MTI) plays a role in shaping the *Arabidopsis* phyllosphere microbiome.

## Cultivation-independent analyses of functional characteristics of rice endophytes

P36

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To study the impact of plant genotype and plant growth stage on the microbial community composition and function, four different rice varieties belonging to *Oryza sativa* L. were harvested at four time points of the rice life cycle. Community analyses are performed based on 16S rRNA gene using DNA and RNA extracted from root as well as from the stem part of the plant. Furthermore, to identify important functions of rice endophytes relevant for nutrient cycling, we screen for the key functional genes.

In view of the fact that methane (CH<sub>4</sub>) is a significant greenhouse gas, one part of this study is focused on methane-oxidizing communities. A microbial diagnostic microarray targeting *pmoA* gene (particulate methane monooxygenase) as well as sequence analysis is applied for specific detection of methane oxidizing bacteria (MOB) down to the species level.

Global rice agriculture relies on extensive use of herbicides which represents environmental hazard. Endophytes have been reported to take part in the degradation of organic pollutants. For that reason, we extracted DNA and RNA from rice plants harvested instantly before and after herbicide treatment to investigate the abundance and activity of genes involved in pollutant degradation. Community analysis is performed to observe the response of the endophytic community upon herbicide treatment.

## Friend or foe: Genetic and functional characterization of plant endophytic *Pseudomonas aeruginosa*

P37

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Endophytic *Pseudomonas aeruginosa* strain BP35 (PaBP35) was originally isolated from aerial shoots of blackpepper grown in the Western Ghats of Kerala, India. Strain PaBP35 was shown to provide significant protection to blackpepper against infections by *Phytophthora capsici*. For implementation in disease management programs, several genetic and phenotypic features of PaBP35 were investigated including endophytic behavior, phylogeny and toxicity to mammals. The results showed that PaBP35 efficiently colonized the blackpepper cut-shoots and displayed a typical spatiotemporal pattern in its endophytic movement with concomitant suppression of *Phytophthora* rot. CLSM imaging further revealed high endogenous populations of PaBP35::*gfp2* in tomato plantlets, exemplifying its endophytic behavior in other plant species. Genotyping based on MLST and CGH analysis revealed that PaBP35 clusters with *P. aeruginosa* strains from environmental and clinical habitats. Like other *P. aeruginosa* strains, PaBP35 exhibited resistance to multiple antibiotics, grew at 25-41 °C and produced rhamnolipids, HCN and phenazines. PaBP35 displayed strong cytotoxicity on mammalian A549 cells indicating its proficiency in type II secretion effectors. Coupled with pathogenicity in a murine airway infection model, we conclude that this plant-endophytic strain is as virulent as clinical *P. aeruginosa* type strains. Safety issues related to the selection and practical implementation of plant-endophytic bacteria for crop protection in developing countries will be discussed.

## A role for the plant immune system in shaping root-associated microbial communities

P38

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While it is clear that plants act to assemble specific microorganisms into their root-associated microbial communities, the extent and means by which plants control these interactions largely remains unexplored. To identify host mechanisms responsible for shaping the composition of these different microbial communities, we examined a role for the plant immune system. Our hypothesis is that if a plant cannot properly sense or respond to its microbial environment; it cannot act to control the ability of specific microorganisms to colonize its root-associated communities. To address this question, we have determined the microbial composition of the rhizosphere and endophytic compartment for several classes of immune system mutants in *Arabidopsis thaliana*, including both hypo- and hyperresponsive plants using deep 16S sequencing. Preliminary analysis indicates that plants with altered phytohormone production have dramatically altered root-associated microbial communities from those of their wild-type controls. Community differences in these mutants were also observed using PCR independent methods, such as metagenomic sequencing and hybridization studies. Together these results suggest that the control of phytohormone pathways are among the mechanisms hosts use to shape the root-associated communities of *Arabidopsis*.

## Ecology of diazotrophs associated with *Miscanthus*

P39

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Strategies that minimize anthropogenic energy inputs and promote N fixation are needed to achieve the goal of sustainable production of bioenergy feedstocks. Diazotrophs associated with non-legume biofuel candidates have the potential to enhance sustainable production. However, knowledge of diazotroph communities associated with gramineous crops is limited. **The aim of this study is to identify diazotroph genera associated with *Miscanthus*, and investigate ecological drivers of diazotrophic communities.** *nifH* pyrosequencing was used to identify diazotroph taxa associated with native and agricultural *Miscanthus*. *nifH* T-RFLP coupled with ecological analyses was used to identify ecological drivers. Composition and relative abundance of diazotrophs differed among endophyte and rhizosphere samples, and was influenced by plant species and soil chemistry. *Proteobacteria* was the most abundant phylum in both endophyte and rhizosphere samples. *Burkholderia*, *Bradyrhizobium* and *Xanthobacter* were the most abundant diazotroph genera in both endophyte and rhizosphere samples, representing 30 - 50% of sequences. Understanding drivers of the diazotroph community associated with biofuel feedstocks will enable us to better utilize the nitrogen fixation process and lead to sustainable agronomic practices for biofuel production.

## Growth promoting action of the soil bacterium *Raoultella terrigena* on *Arabidopsis thaliana*

P40

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Plant growth-promoting rhizobacteria (PGPR) colonize the rhizosphere and live in close association with the roots of many crop species such as wheat or barley. Besides the fixation of atmospheric nitrogen and release of nitrogen to plant roots, PGPR might counteract pathogenic infections or produce and release phytohormones. During the course of the EU project RHIBAC ('Rhizosphere bacteria for reduced fertilizer input in wheat'), a rhizosphere bacterium *Raoultella terrigena* was isolated. *Raoultella terrigena* reliably promotes the growth of *Arabidopsis thaliana* when grown on standardized agar medium. However, growth promotion depended on the extent and forms of nutrients being used in agar medium. Initial data suggested a considerable genetic variability for the responsiveness of *A. thaliana* lines to *R. terrigena*. To investigate plant genetic factors underlying growth promotion by *R. terrigena*, a forward genetic approach is conducted by using 19 *A. thaliana* accession lines and their intercrossed recombinant inbred lines, which are designed for association map-based cloning (MAGIC lines). In addition, a transcriptome approach had been conducted using root and shoot tissues from *R. terrigena*-inoculated *A. thaliana* plants grown on agar medium. Combining both approaches it is aimed at identifying *A. thaliana* genes determining the extent of the growth promotion conferred by *R. terrigena*.

## Exploring cross scale interactions within and beyond microbiomes to advance understanding of plant adaptation

P41

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Plants are commonly viewed as discrete entities at the base of the food web, in recognition of their role as primary producers. This perspective restricts knowledge of plant adaptation to understanding of *plant* cellular and genetic processes. Data illustrating microbiome diversity along the plant-soil interface are revealing infinite, poorly explored pathways through which diverse organisms, including plants, bacteria, fungi, and microfauna, might interact to influence plant survival and adaptation. Novel observations made through advanced imaging and metagenomics can best be interpreted relative to host adaptations and habitats by exploring these community associations across spatial and temporal scales. Experiments that explore such complex, cross-scale interactions are obligately multidisciplinary, and require integration of many data types.

Our Plant-Microbe Database was designed to consolidate metagenomic data describing microbiomes with metadata defining the host plants, soils, location, and ecological sites on which the microbiomes developed. We have included tools to assist with management of 454 pyrosequencing data, and of metadata associated with nematodes and microarthropods that also colonize plants and soils. This ability to relate data from diverse ecosystem components is a powerful tool crucial for advancing holistic understanding of biotic interactions throughout entire energy webs that influence and are influenced by plants.

## Defining and isolating the core *Arabidopsis thaliana* root microbiome

P42

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Land plants associate with a root microbiota distinct from the microbial community present in surrounding soil. The microbiomes colonizing the rhizosphere and the inside of the root contribute to plant growth and development, crop productivity, carbon sequestration and phytoremediation. Natural colonization of the root occurs in the presence of a robust plant immune system, suggesting the plant can finely discriminate pathogens from mutualists, but genetic principles governing the winnowing of the complex soil microbial community into the root-specific endophytic population are largely unknown. A multiplexed amplicon pyrosequencing survey of the bacterial 16S rRNA gene in >600 individual *Arabidopsis thaliana* plants grown in natural soils demonstrated the effect of geochemically distinct soils, proximity to the plant, developmental stage, and plant genotype on the microbial community. To reduce the complexity of the system and for greater control of microbial inoculum, we identified a set of diverse root-enriched bacteria from the pyrosequence study through a culture-based isolation effort and gave this synthetic community to otherwise sterile plants of varied genotypes grown in an artificial substrate in 12-well culture plates, with plans to quantify community membership for each plant. Results of the pyrosequence study and the synthetic community study will be discussed.

## Effects of genes, environment, and their interaction on secondary chemistry and root microbiome

P43

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The perennial herb *Boechera stricta* occupies diverse habitats throughout its native range, and previous work has demonstrated local adaptation to site of origin. We hypothesized that the *B. stricta* root microbiome is [1] influenced by genes, environment, and their interaction, and [2] sensitive to quality and quantity of glucosinolates—defensive chemicals that deter pathogens in *Arabidopsis*. We grew genotypes from 5 wild populations of *B. stricta* in undisturbed sites near the original populations in Idaho, USA. To test effects of glucosinolates, we also planted near-isogenic lines segregating for a locus controlling glucosinolates. To map loci influencing microbiome assembly, we planted recombinant inbred lines of *B. stricta* in the same common garden. Plants were harvested after 2-3 years in the field and their root microbiomes and glucosinolates were analyzed. To isolate effects of soil properties and biota from other environmental factors, we repeated the experiments in the greenhouse using soil collected from each common garden. Association of microbiome with environment, genetic variation, and glucosinolate content will be discussed.

## Activities of 9-lipoxygenase and alpha-dioxygenase oxylipins in protecting plants from pathogen infection

P44

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Plant oxylipins are a class of lipid signalling molecules with a critical role in protecting plants against pathogen attack. Recent studies demonstrate the participation of the 9-LOX and alpha-DOX oxylipin pathways in the defence mechanisms activated by *Arabidopsis* following infection by hemibiotrophic bacteria, in which these enzymes collaborate to achieve full resistance against virulent strains. We found that these oxylipin pathways participate in the three layers of defence -pre-invasion, apoplastic and systemic defence- triggered by plants to prevent *Pseudomonas syringae* pv *tomato* DC3000 infection. In these responses, oxylipins were found to act as regulators of oxidative stress and hormone homeostasis. Our studies also showed high 9-LOX and alpha-DOX activity in roots of untreated *Arabidopsis* plants. Studies with mutants deficient in alpha-DOX and 9-LOX activity and signalling indicated that these oxylipin pathways might participate in plant defence mechanisms against root pathogens, a process that remains poorly understood. These results support our interest in examining 9-LOX and alpha-DOX oxylipin pathways functions in plant defence as well as in identifying the molecular components that mediate their activity. Understanding the mode of oxylipin action at distinct layers of defence and tissues will provide further insight into the plant machinery that function to avoid pathogen invasion and proliferation.

## Environmental regulation of flagellar synthesis in the rhizobacterium *Pseudomonas fluorescens* F113

P45

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Flagella mediated motility, an important trait for competitive rhizosphere colonization and biocontrol ability is tightly regulated in *Pseudomonas fluorescens* F113. We have previously shown that swimming motility is repressed independently by the GacA/GacS system and by SadB through downregulation of the *fleQ* gene, encoding the master regulator of the synthesis of flagellar components. Here we show that both regulatory pathways converge in the regulation of transcription and possibly translation of the *algU* gene, which encodes a sigma factor. AlgU is required for multiple functions, including the expression of the *amrZ* gene which encodes a transcriptional repressor of *fleQ*. Gac regulation of *algU* occurs during exponential growth and is exerted through the RNA binding proteins RsmA and RsmE but not RsmI. RNA immunoprecipitation assays have shown that the RsmA protein binds to a polycistronic mRNA encoding *algU*, *mucA*, *mucB* and *mucD*, resulting in lower levels of *algU*. We propose a model for repression of the synthesis of the flagellar apparatus linking extracellular and intracellular signalling with the levels of AlgU and a new physiological role for the Gac system in the downregulation of flagella biosynthesis during exponential growth.

## The smelly road - how *Bacillus* sp. B55 promotes plant growth

P46

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Plants have intimate relationships with soil microbes that through a variety of mechanisms improve their host's growth and fitness. *Bacillus megaterium* is a natural endophyte of *Nicotiana attenuata* (coyote tobacco) growing in native soils. The isolate B55 was found to have dramatic plant growth promoting (PGP) effects on wild type and transgenic plants impaired in ethylene (ET) perception (*35S-etr1*), the genotype from which B55 was first isolated. B55 not only improves *N. attenuata* growth under *in vitro*, glasshouse and field conditions, but also 'rescues' many of the deleterious phenotypes associated with ET insensitivity, by increasing growth and survival of the hampered *35S-etr1* plants in nature. *In vitro* studies show that volatile organic compounds (VOCs) emitted by B55 promote the growth of seedlings. In particular, dimethyl disulfide (DMDS) is produced by the bacteria. <sup>35</sup>S-labeling experiments demonstrate that a sulfur containing VOC, most probably DMDS, is taken up by the seedlings and the sulfur incorporated into proteins. Application of synthetic DMDS to seedlings underlined its PGP effect: seedling surface area and levels of chlorophyll, methionine and glutathione were all increased compared to the controls. Our results indicate that DMDS is involved in bacterial PGP, likely by enhancing plant sulfur nutrition.

## Contrasting bacterial and functional diversity between soybean rhizosphere and bulk soil in Brazilian Amazonian region

P47

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The Southeast Amazon region is considered the largest agricultural frontier in the world, where native forests are converted into soybean crop fields, a fact that highlights the social and economic importance of this system to Brazil. The taxonomical and functional structure of soil microorganisms are influenced by various biotic and abiotic factors including soil properties and plant species. Using high-throughput next generation 454 pyrosequencing, we investigated the phylogenetic and functional diversity of microbial communities colonizing the rhizosphere and the bulk soil associated to soybean crop field. From 24 independently soil samples from greenhouse mesocosms experiments, we obtained over than 3 million shotgun metagenomics sequences from soil of 1st and 5th years of cultivation. Multivariate analysis of the relative abundance of different phyla and functions revealed a net differentiation of the bacterial and functional communities present in the rhizosphere and the bulk soil. Significantly more Actinobacteria inhabited the rhizosphere when compared with surrounding soil. Likewise, Nitrogen and Sulfur metabolism functions were correlated to rhizosphere. In addition, the chronosequence (one and five years of cultivation) also presented differences, suggesting an influence over time. These data suggest a rhizosphere effect over the soil community, not only taxonomically, but also in a functional level.

## The dual role of plant-associated fungi in the biodiversity-productivity relationship

P48

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The positive correlation between biodiversity and productivity of grasslands has so far been attributed to resource complementarity of plant species. Here, we examine whether this phenomenon of overyielding can be explained by a more recently proposed hypothesis: higher productivity of more diverse plant communities is determined, in part, by the composition of rhizosphere fungal communities. An experiment using soils trained with soil biota of four different plants and a proportional mixture of these soils showed that in monocultured soils, plant productivity was reduced compared to mixture soils, but restored by soil sterilization. The results also showed that growth of the grass species *Anthoxanthum odoratum* was substantially promoted when growing on *Leucanthemum vulgare* soil. Preliminary experiments suggest that specific members of the soil-borne fungal community contribute to this enhanced productivity. To unravel both the deleterious and growth-promoting effects of soil-borne fungi on plant productivity in a biodiversity context, two different approaches are pursued. The diversity and abundances of fungi in a long-term biodiversity experiment are assessed by metagenomic analyses. Simultaneously, 70 fungi were isolated from roots of the different plant species, classified by ITS-sequencing and evaluated for their effects on plant growth. These top-down and bottom-up approaches will reveal whether species-specific networks of interactions among plants and soil fungi are key elements of the biodiversity-productivity phenomenon.

## Characterization of ectomycorrhizal, pathogenic and saprophytic fungi by emission patterns of biogenic volatile organic compounds

P49

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Interactions between plants and soil microorganisms are mediated by the emission and perception of a variety of biogenic volatile organic compounds (BVOCs). Between different groups of organisms BVOCs may act as attractant or repellent signals. BVOCs emitted by fungi may play important roles in the recognition of and communication with plant roots. Our objectives are to characterize and compare ectomycorrhizal (EM), pathogenic and saprophytic fungal species based on BVOC emission patterns. BVOC emissions were collected from nine fungal species (four EM, three pathogens and two saprophytes) using the stir bar sorptive extraction method (Twister system, Gerstel) and analyzed by GC-MS. Principle component and cluster analyses revealed that the fungal species could be separated by their distinctive BVOC emission pattern. This specific emission pattern could play important roles in varied interspecies recognition and interactions of fungi with other organisms such as plants. Ongoing research addresses the changes in emission patterns in presence of roots.

## Interaction between Arabidopsis and natural bacterial isolates

P50

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Endophytic bacteria reside inside plant tissue and can have beneficial effects on plants. The underlying mechanisms regulating interactions between plant and microbe remain unclear. There are long-standing fundamental questions regarding the true diversity of endophytic bacterial populations and how they colonize and co-exist with plants in peace while exerting beneficial effects on the hosts. Using culture-independent approaches including 16S rRNA clone-library sequencing and the 16S rRNA microarray technology known as Phylochip, we found diverse bacterial communities associated with healthy Arabidopsis roots and leaves. This diversity encompassed 1877 bacterial taxa belonging to 42 phyla. To further investigate the interaction between host and microbes, a culture-dependent approach was undertaken and bacteria were isolated from surface-sterilized *A. thaliana* growing in a variety of soils. This non-exhaustive isolation yielded a total of 56 bacterial species. Regardless of the soil in which plants were grown, some species were consistently found associated with roots; the most common isolates included Methylobacteria and *Microbacterium ginsengisoli*. Current studies are focusing on molecular interactions between *A. thaliana* and the isolated bacteria. We are particularly interested in the role of the plant cell wall and secondary metabolites in colonization and co-existence as well as the interactions between diverse bacteria *in planta*.

## Deforestation in Amazon simplifies bacteria-bacteria-soil interactions (BBSi) and affects verrucomicrobial community

P51

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Network analysis was applied to a barcoded pyrosequencing dataset containing 423,145 sequences of the V3 16S rRNA gene region from discontinuous areas of mature rainforest and adjacent deforested sites located at Brazilian Amazon. The structure of the identified network suggested that the interactions among different phylogenetic bacterial groups and abiotic soil factors were markedly simplified in deforested soils. Furthermore, *Verrucomicrobia* phylum showed to be significantly affected to deforestation in the Amazon forest. Based on qPCR measurements, *Verrucomicrobia* accounted, on average, for 4% and 2% of the total bacterial signal, in forest and deforested soils, respectively. The phylum *Verrucomicrobia* represented on average 5% and 2.5% of the bacterial sequences from the forest and deforested soils, respectively. Among different groups of *Verrucomicrobia*, the *Spartobacteria* was dominant and accounted on average for 83% and 72% of the total verrucomicrobial community for forest and deforested soils, respectively. Principal component analysis based on terminal restriction fragment length polymorphism (T-RFLP) data revealed that verrucomicrobial community structures of adjacent intact forest and deforested soils are different. The findings suggest *Verrucomicrobia* as a model responsive phylum to study the effects of deforestation on bacterial community in Amazon forest soils.

## Insights into soil acidobacterial community based on Amazon soil characteristics

P52

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*Acidobacteria* has been described as ubiquitous and abundant in Amazon soil. However, the ecological characteristics of this dominant group in bacterial community are not known. Taxon-specific quantitative real-time PCR (qPCR) assays and 16S rRNA gene pyrosequencing analysis were applied to study the acidobacterial community in bulk soil samples from croplands and adjacent forests as well as soybean rhizosphere located in Southeastern Brazilian Amazon. Based on qPCR, *Acidobacteria* accounted of 23.25 %, 17.54 % and 14.35 % of the total bacteria, in forest soils, cropland soils and soybean rhizosphere samples, respectively. The phylum *Acidobacteria* represented 28.43 %, 16.32 % and 16.52 % of the sequences generated from forest soils, cropland soils and soybean rhizosphere samples, respectively, with dominance of *Acidobacteria* subgroup Gp1. Subgroups Gp4, Gp6 and Gp7 were significantly higher in cropland soil samples than the remaining samples. Subgroup-level relative abundance of *Acidobacteria* correlated with soil factors linked to soil acidity, such as pH, Al<sup>+3</sup>, Al saturation and base saturation as well as with Ca<sup>+2</sup>, Mg<sup>+2</sup>, Z<sup>+</sup>, P, Fe, Mn and B. The findings indicate that an explanation for the acidobacterial community pattern found in soils from Southeastern Brazilian Amazon is a causal interaction among soil factors.

## Endophytic bacterial communities in the Arctic

P53

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We used molecular and culture-dependent approaches to characterize endophytic bacterial communities of three arcto-alpine plant species (*Oxyria digyna*, *Diapensia lapponica* and *Juncus trifidus*) in the low Arctic fell tundra. Analysis of clone libraries as well as bacterial isolates, collected from seven sampling sites, revealed a high diversity, representing mainly Actinobacteria, Bacteroides, Firmicutes, Acidobacteria and  $\alpha$ -,  $\beta$ - and  $\gamma$ - proteobacteria. Taxonomic distributions of the culturable isolates as well as the clone libraries were dependent on host plant species as well as on sampling site properties. Significantly, several bacterial genera, including *Burkholderia* and *Sphingomonas* were tightly host plant specific. The isolated bacteria were well adapted to growth at low temperatures and retained e.g. chitinase and protease activities at +0 - +4°C. This preference for cold temperature habitats was also reflected in the phylogenetic affiliation of the isolates and clone libraries, with the closest relatives in public databases often representing isolates from cold environments.

We are currently extending these studies by addressing the diversity of plant-associated soil and endophyte populations in relation to climate zone and to plant species by molecular community fingerprinting and pyrosequencing of bulk soil, rhizospheric and endophytic communities.

## Phosphate solubilisation and gluconic acid production by endophytic bacterial strains and ability to promote plant growth in oil seed rape (*Brassica napus*)

P54

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Several studies have shown that the release of low molecular weight organic acids is a major mechanism for solubilising insoluble phosphate by phosphate solubilising bacteria (PSB). The production of gluconic acid during inorganic phosphate solubilisation in conjunction with liberation of phosphate and the influence on plant growth as a function of phosphate solubilisation by endophytic strains was analysed. Solubilisation of  $\text{Ca}_3(\text{PO}_4)_2$  in National Botanical Research Institute's Phosphate (NBRIP) growth medium varied among the endophytes with P-liberated ranging from 1109.33  $\mu\text{g}/\text{ml}$  to 67.3  $\mu\text{g}/\text{ml}$ . In all cases, the final supernatant had a significant pH decrease and this had a direct relation to P-liberated. High Performance Liquid Chromatography (HPLC) analysis of the culture filtrate to quantify gluconic acid produced by the strains ranged from 33.21 $\pm$ 2.34 mg/ml to 2.2  $\pm$ 0.18 mg/ml. The results suggest that acidification was the main strategy for solubilising phosphate. In this study, a clear relationship was observed between supernatant acidification and P solubilisation from  $\text{Ca}_3(\text{PO}_4)_2$ . However, no significant difference was observed for key growth parameters in oil seed rape between treatments. The result of this study indicates in planta expression of P solubilisation traits may be more complex than those *in vitro* studies.

## Arabidopsis root hairs growth reaction in response to bacteria

P55

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Recently, a role for exocyst subunits isoforms EXO70B2 and H1, and based on their interactions with other exocyst subunits probably for the whole complex, in plant foliar defense reaction was found (Pecenкова *et al.*, 2011). We have observed that Arabidopsis seedlings' roots exposed to pathogenic bacteria change their architecture similarly to what was described for plant growth-promoting rhizobacteria - the primary root growth is arrested, while the growth of the root hairs is enhanced. We have analyzed these reactions using knock-out mutants in EXO70A1, B2 or H1 as well as mutants impaired in pathogen and ethylene perception. Pathogen perception mutants, when compared to WT, were less sensitive to primary root growth inhibition, however, there was no difference between root hairs growth response between pathogen perception mutants and WT. On the other hand, all exocyst mutants studied showed significant decrease in both primary root growth arrest and in root hair lengths after pathogen treatment. We will present advances in our analysis of this interaction.

## Functional differentiation of ectomycorrhizal fungal species for acquisition of litter-derived nitrogen

P56

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The functional diversity of composed natural EM communities for nitrogen (N) acquisition from leaf litter is unknown. The goal of this study was to investigate the interspecific accumulation of litter-derived N in EM root tips associated with different fungal species in comparison with roots and soil.

<sup>15</sup>N labeled leaf litter from beech (*Fagus sylvatica*) was buried in mesh bags, which were inaccessible to roots, in soil in an old-growth beech forest. The appearance of the label in soil, roots and EM surrounding the mesh bags was measured during an exposure time of 18 months. Four months before the final harvest, half of the mesh bags were removed to distinguish between N-uptake from litter- and soil-derived pathways, respectively. The <sup>15</sup>N was retrieved first in EM root tips, then in roots and finally in soil. Seven EM fungal species were present on all sampling dates and revealed clearly distinct temporal patterns and enrichment of <sup>15</sup>N. The clear temporal pattern of <sup>15</sup>N accumulation shows that EM adjacent to litter patches outcompete other soil microbes for litter-derived N. Our study reveals functional differentiation of EM communities with regard to soil- and litter-derived N and with regard to N accumulation.

## The influence of arbuscular mycorrhizal fungal communities on drought tolerance of a native Kenyan grass

P57

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Because drought is a ubiquitous stress on plant communities numerous adaptations, both physical and physiological, have evolved to help plants withstand periods of drought. Plants also rely on symbionts for protection against drought. Arbuscular mycorrhizal fungi (AMF) can play an especially important role in allowing plants to survive drought. However, all AMF communities may not provide equal amounts of protection. We examined the effects of two distinct AMF communities on drought tolerance of a native Kenyan grass. Communities were collected from on and off termite mounds because these areas have pronounced differences in edaphic properties that lead to very different water relations. Overall, we found that AMF drought stressed plants added more leaves post-drought and had more aboveground and belowground biomass than non-AMF drought stressed plants. In addition, plants inoculated with AMF collected from termite mounds performed better overall than plants inoculated with fungi from off-mound areas. On average, drought-stressed plants harboring fungi from termite mounds produced 18% more aboveground biomass and 45% more belowground biomass than those with fungi from off-mound areas. These findings highlight the important ecological role that AMF play in mitigating plant stress and they indicate that different mycorrhizal communities offer different degrees of physiological advantage.

## Unraveling the vineyard's microbiome using a metagenomic approach

P58

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Grapevine (*Vitis vinifera*) is one of the most widely cultivated fruit crops in the world. As a plant, is naturally colonised by a wide variety of microorganisms, which interact with it and play a major role in its growth. The structure of this microbial community is mainly affected by spatial and temporal factors. In this study, we have extensively characterized the natural microbiome present on grapevine during the growth vegetative cycle using a metagenomic approach. The analysis revealed a surprising complex microbiome associated with *V. vinifera*. Our samples are mainly characterized by the dominance of the eukaryotic *Aureobasidium pullulans* and the prokaryotic *Enterobacteriaceae*. Indeed, during the vegetative cycle, there is an increase on the microbiome biodiversity even though the notable influence of the vineyard's chemical treatments on microflora density. Overall, the simultaneously study of eukaryotic and prokaryotic population present on grapevine allowed to both unravel a great microbial biodiversity and infer about the interactions between plant-microbe communities. The application of Next Generation Sequencing (NGS) methodology represented an enormous breakthrough and allowed us to unveil the natural grapevine microbiome.

This work is supported by the InovWine project (Ref. n.º 11498), which is financed with funds from FEDER through the QREN program.

## Functional analysis of Mycorrhiza induced Small Secreted Proteins (MiSSPs) from the mutualistic fungal symbiont *Laccaria bicolor* in controlling host colonization and host specificity

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Soils contain a multitude of fungi with vastly divergent lifestyles from saprotrophic to mutualistic and pathogenic. The recent release of many fungal genomes has led to comparative studies that consider the extent to which these lifestyles are encoded into the genome. Boreal and temperate forest ecosystems rely on ectomycorrhizal (ECM) symbiosis for trees nutrition, productivity and stress resilience. Despite their ecological significance, very little is known about the mechanisms by which ECM fungi, which are thought to have evolved from saprotrophic ancestors, communicate with their host plants or act to structure the surrounding microbiome. Using transcriptomic approaches on roots colonized by the ECM fungus *Laccaria bicolor*, a number of effector-like Mycorrhiza induced Small Secreted Proteins (MiSSPs) encoded by the ECM partner were identified (Martin et al., 2008) and are thought to play an integral role in establishment of symbiosis and controlling organisms populating the rhizosphere. Our group has been involved in the on-going characterization of a number of highly expressed MiSSPs when *L. bicolor* is colonizing a variety of hosts (Plett et al., 2011). We will present data demonstrating that (i) proper expression of these SSPs are required for symbiosis development, (ii) identify which plant compartment/proteins are targeted by MiSSPs and (iii) present our current model by which we think that these proteins negotiate symbiosis and alter the local fungal community.

## Leaf microbiota in an agroecosystem: spatiotemporal variation in bacterial community composition on field-grown lettuce

P60

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The presence, size, and importance of bacterial communities on plant leaf surfaces are widely appreciated. However, information is scarce on how their composition changes along geographical and seasonal scales. We collected 106 samples of Romaine lettuce from commercial production regions in California and Arizona during the 2009/2010 crop cycle. Total bacterial populations averaged to  $10^5$ - $10^6$  per gram of tissue, whereas counts of culturable bacteria were 1-2 orders of magnitude lower. Pyrosequencing of 16S rRNA gene amplicons from 88 samples revealed *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* as the most abundantly represented phyla. At the genus level, *Pseudomonas*, *Bacillus*, *Massilia*, *Arthrobacter*, and *Pantoea* were the most consistently found across samples. The foliar presence of the lettuce pathogen *Xanthomonas campestris* pv. *vitiens* correlated positively with the relative representation of the genus *Alkanindiges*, but negatively with *Bacillus*, *Erwinia* and *Pantoea*. Summer samples showed an overrepresentation of Enterobacteriaceae sequences and culturable coliforms compared to winter. The distance between fields or the timing of a dust storm, but not Romaine cultivar, explained differences in bacterial community composition between fields. This study offers new insights into the extent of variability in bacterial community composition on plant leaves as a function of time, space, and environment.

## The genomic sequence of *Pseudomonas fluorescens* F113 reveals multiple traits important for the rhizosphere environment

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*Pseudomonas fluorescens* F113 is a biocontrol and plant growth promoting rhizobacterium isolated from the sugar-beet rhizosphere. This bacterium has been genetically modified for PCBs rhizoremediation and is used as a model strain for rhizosphere colonization. Sequencing and annotation of its genome has shown that besides a core genome very similar to other strains belonging to the *P. fluorescens* complex, it contains an unusual number of features related to the adaptation to the rhizosphere environment and to the interaction with eukaryotic organisms. Traits include genes required for: denitrification, diterpenes metabolism, production of a second flagellar apparatus, three chemotaxis systems, an animal pathogen-type Type Three Secretion System, a novel antibiotic and toxins likely to be targeted to insects. Most of these traits seem to be acquired through horizontal transfer from soil/rhizosphere bacteria belonging to the Proteobacteria. Although the genome of *P. fluorescens* F113 shows that this strain belongs to the *P. fluorescens* complex of species, it shows a striking abundance of genes encoding traits that are not usually found together in previously sequenced genomes within this group. Some of these traits are unique among sequenced pseudomonads. Analysis of the genome also shows that horizontal gene transfer from related soil/rhizosphere bacteria has been an important force in the shaping of this genome for rhizosphere adaption and interaction with other organisms.

## Variation in local carrying capacity and the individual fate of bacterial colonizers in the phyllosphere of green snap bean (*Phaseolus vulgaris*)

P62

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We demonstrate that the term ‘carrying capacity’, as it is commonly used in microbial ecology, needs to be understood as the sum of ‘local carrying capacities’ to better explain and predict the course and outcome of bacterial colonization of an environment. By reconstructing the contribution of individual bacterial immigrants to observed changes of a total population we reveal that a leaf represents an environment where the fate of individuals is determined by local carrying capacity. We were able to infer the relative contribution of micro-niches with different carrying capacities to the leaf under study. The obtained data suggest that the leaves offered three kinds of micro-niches that feature low, intermediate or high carrying capacities. We estimated that niches with low or intermediate carrying capacity made up 49% of the leaf each, while niches with high carrying capacity made up 2% of the total leaf surface.

Our findings contribute to a bottom-up understanding of leaf surface colonization, which includes a quantifiable role of chance in the experience at the individual level and in the outcome at the population level.

## The rhizobacterium *Pseudomonas fluorescens* F113 escapes protozoan predators by Gac-dependent and Gac-independent mechanisms

P63

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Rhizobacteria, while interacting with the plant host, are exposed to predation by bacteriophagous invertebrates such as protozoa. Therefore, rhizospheric bacteria may have evolved molecular mechanisms to face this ecological pressure. We tested the ability of the protozoan *Acanthamoeba polyphaga* to graze on *Pseudomonas fluorescens* F113. *A. polyphaga* was unable to feed and multiply on *P. fluorescens* F113 wild-type. However *gacA* or *gacS* mutants (*P. fluorescens* F113 derivatives lacking secondary metabolites) supported amoebal growth but did so to a lesser extent than a harmless *Escherichia coli* strain. At the cellular level, *A. polyphaga* in co-culture with F113 wild-type emitted long filopodia prior to cell death, and interaction with the *gacA* mutant showed similar effects of cytoskeletal changes. Our results indicate that *P. fluorescens* F113 possesses Gac-dependent and Gac-independent mechanisms of toxicity towards *A. polyphaga*.

## Bacterial endophytes in wheat (*Triticum aestivum*): isolation, characterisation and bio-prospecting

P64

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Endophytic bacteria colonise the internal tissue of living plants without causing symptoms of disease. Wheat, an important agricultural crop worldwide, has not yet been subject to a comprehensive survey of root or shoot bacterial endophytes. We are using material from the Broadbalk long-term experimental plots at Rothamsted Research, UK, to complete a comparative study of bacterial endophyte communities in winter wheat (*Triticum aestivum* cv. Hereward) under contrasting fertiliser regimes. This work aims to determine whether agricultural inputs alter abundance and diversity of bacterial endophytes and whether wheat endophytes form a selected subset of wheat rhizobial and soil microbiota. Further work also aims to explore the consequences of endophyte colonisation on the host plant and to identify endophyte niches within plants. A combination of culture and molecular techniques were used for isolation and identification of endophytes. Endophyte populations were profiled using 16S rRNA gene RFLP analysis and unique profiles were selected for 16S rRNA gene sequencing. Unique endophytes will be subject to further physiological and morphological characterisation and screening for biochemical traits such as indole acetic acid production or for expression of potentially beneficial genes such as nitrogen fixation (*nifH*). We will present results concerning endophyte identity, abundance and properties.

## Identification and cultivation of Rhizobiales as key members of the Arabidopsis root-inhabiting microbiota

P65

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Plant roots form intimate associations with soil derived bacteria living attached to the root or even within the root. Such bacterial communities associated to eukaryotic hosts present an extended genotype of up to 100 times more genes than the host's genome itself, providing a largely unknown basis of modulating the host's performance.

We profiled natural soil- and root-inhabiting bacterial communities by pyrosequencing of the 16S rRNA gene. Roots of *Arabidopsis thaliana* grown in natural soil are preferentially colonized by probably soil-derived Bacteroidetes, Chloroflexi, Actinobacteria and Proteobacteria, including a number of Rhizobiales. Based on the generated sequencing information, we could cultivate several of the previously identified key members of the root-inhabiting microbiota. Isolated Rhizobiales were RFP-tagged and are able to re-colonize Arabidopsis roots in *in vitro* experiments allowing us to study colonization effects in simplified systems. Moreover, we sequenced their genomes to gain insight into the genetic potential of these host-microbe interactions. Both culture-independent and -dependent approaches will help to understand the link between soil-derived root-inhabiting bacteria and plant performance.

## Fungal endophyte infection and host genotype jointly modulate host response to an aphid-transmitted viral pathogen

P66

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Interactions between microbial pathogens and their hosts may be modified by other microbes, including mutualistic symbionts. B/CYDVs are aphid-transmitted, host-generalist plant viruses that can negatively impact natural and agricultural ecosystems. Endophytic fungal symbionts are vertically transmitted intercellular fungi that form obligate associations with grasses and relieve both biotic and abiotic stresses through the accumulation of alkaloids. In the greenhouse, we factorially manipulated virus infection and endophyte presence with two genotypes of tall fescue (*Schedonorus phoenix*), one in which the endophyte produces alkaloids (KY 31) and one that possesses a novel endophyte that does not produce alkaloids (PDF). Endophyte infection decreased overall plant biomass for the KY 31 genotype but tended to increase overall plant biomass for the PDF genotype. Furthermore, endophyte infection altered plant response to virus infection. Across endophyte status and genotype, virus infection decreased the root fraction of endophyte-free plants, but when endophyte infection is taken into account, root allocation for virus infected plants increased. Overall, this work indicates that to understand the full impact of B/CYDV infection in plant hosts, we must not only consider the mutualists with which they associate but the origin of that association as well.

## Community structure of mycophagous bacteria in the plant rhizosphere

P67

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Despite the ecological importance of interactions between fungi and bacteria we have only little knowledge about the ecology of mycophagous (fungi eating) bacteria in soil ecosystems. We are currently running an experiment determine how communities of mycophagous bacteria differ with fungal communities whilst comparing dissimilar plant rhizospheres. We will inoculate fungi of 3 major groups (Basidiomycota, Ascomycota, Zygomycota) with a bacterial suspension that originates from different plant rhizosphere soils. Subsequently, actively feeding bacteria will be sequenced and cultured. Relating abundances of different mycophagous bacteria to host fungi and plant species is going to allow us to evaluate the role of mycophagous bacteria in rhizosphere food webs in order to understand if plants are able to shape the community of mycophagous bacteria or if is solely the host fungus that selects. Besides that, understanding establishment of mycophagous bacterial communities in plant rhizospheres is an important step in the search for biocontrol bacteria against plant diseases of fungal origin.

## Herbs: acting as probiotics?

P68

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Probiotics have recently been broadly defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Such useful bacteria are contained for example in yogurt, lactobacillary beverages or fermented foods and have been medically recognized.

Herbs are known to act in different ways, as spices in food but also in medication of intestinal infectious diseases. The mechanisms on which the hearbal medication acts have only recently been proposed. In different herbs are specific ingredients known i.e. to suppress harmful microorganisms by producing essential oils or glucosides or support health via vitamins and hormones. Our additional hypothesis is improving the balance of intestinal bacterial flora by the highly diverse herbs inhabiting bacterial community probably acting as probiotics. Therefore, eight herbs with contrasting composition of ingredients were grown in greenhouse for 6 weeks. Plant phyllosphere bacterial community composition and their functional diversity were measured using community level physiological profiling, PCR-DGGE, sequencing and quantitative real-time PCR.

Bacterial counts were shown to be in the same order of magnitude as the probiotic bacteria *Lactobacillus* or *Bifidobacterium* were administered. Investigated herbs were colonized by significantly contrasting bacterial communities. Their importance and functions in health care foods will be evaluated.

## Multiple approach at gene, genome and transcriptome levels for deciphering biological mechanisms of soil suppressiveness of a plant root disease

P69

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Take-all of wheat is a root disease caused by the soilborne fungus *Gaeumannomyces graminis* var. *tritici*. The reduction of such disease in suppressive soils can be explained by the shift in pathogen populations (Lebreton *et al.* 2007) and the stimulation of microbial antagonism (Sanguin *et al.* 2009). To know how some rhizobacteria can either influence the dynamic of fungal pathogen populations, the fungal root colonization and fungal pathogenesis, we selected a rhizobacteria strain, *Pseudomonas fluorescens*, belonging to a subgroup of bacteria representative of a disease reduction step (Frey-Klett *et al.* 2011). Combining multiple transcriptome analysis based either on all the bacterial genome or on candidate fungal and plant genes, we monitored the tripartite interactions between roots, bacteria and mycelium fungus. With the help of different appropriate controls, we demonstrated the importance of the fungus to trigger specific bacterial responses related to oxidative stress resistance and involving specific secretion systems (Barret *et al.* 2009). The antagonistic bacterial strain acts through the alteration of fungal pathogenesis and probably through the activation of plant host defenses (Daval *et al.* 2011).

## Composition of root-associated bacterial communities of *Arabidopsis thaliana* and its relatives

P70

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Plants touch with their roots one of the richest microbial ecosystems on earth and engage at this contact zone in intimate associations with a multitude of mainly commensalistic and mutualistic microbes. Root-associated bacterial communities (RABCs) are markedly different from the surrounding soil microbiota indicative of specific colonization events. Little is known about composition principles of RABCs and microbial services they provide to the plant. The composition of RABCs was determined by pyrosequencing of amplicons of the taxonomically informative 16S rRNA gene. Roots of the model plant *Arabidopsis thaliana*, grown in natural soils, are preferentially colonized by Proteobacteria, Bacteroidetes, Chloroflexi and Actinobacteria. Comparing root communities of *A. thaliana* with the phylogenetically closely related Brassicaceae species *Arabidopsis lyrata* and *Cardamine hirsuta* identified a core of Brassicaceae RABCs possibly satisfying the common host needs. This comparison also revealed host species-specific RABC members that are of different taxonomic identity. Species-specific RABC subunits of *Arabidopsis* genera preferentially contain bacteria of the phylum Chloroflexi whereas *C. hirsuta* hosts different Proteobacteria. This observation indicates that the composition of RABCs is linked to the evolutionary divergence time of the host species.

## Genetic responses in roots of a woody species upon colonization by a biocontrol endophytic bacterium: the olive-*Pseudomonas fluorescens* PICF7 case

P71

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Information concerning processes underlying the interaction between beneficial bacteria and woody plants is still very limited and totally absent in the case of olive (*Olea europaea* L.). We aimed to elucidate genomic responses occurring during the colonization of olive roots by the indigenous endophyte *Pseudomonas fluorescens* PICF7, an effective biocontrol agent against Verticillium wilt of olive (VWO). A Suppression Subtractive Hybridization cDNA library, corresponding to up-regulated genes, was generated from roots of olive plants (cv. Arbequina) inoculated with strain PICF7, enabling the identification of some 450 EST sequences differentially expressed during this interaction. Computational analysis showed a number of transcripts involved, among other processes, in plant defence response to different stresses (i.e., PAL, acetone cyanohydrin lyase, lipoxygenase,  $\beta$ -1,3-glucanase, endochitinases or lignin-forming peroxidases). Similarly, diverse transcription factors such as bHLH, GRAS1, ARF2 and SA-responsive WRKYs were shown to be induced in olive roots after PICF7 inoculation. Interestingly, 19% of detected olive transcripts corresponded to unidentified genes. Real-time quantitative PCR confirmed the induction of some of the aforementioned genes. Thus, the beneficial endophytic strain *P. fluorescens* PICF7 seems to trigger a broad array of defence responses in olive root tissues which can be responsible of its VWO biocontrol activity.

## Metabolic root exudate profiles of *Arabidopsis thaliana* MAGIC lines

P72

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Metabolite profiles of root exudates are influenced by endogenous (genetic) and exogenous (soil, nutrients, stress) factors. We established a hydroculture system to grow the 19 parent accessions of the Multiparent Advanced Generation Inter-Cross (MAGIC) *Arabidopsis thaliana* lines and to collect the media in a sterile environment. The media of each accession at 3 timepoints were measured with GC/MS and LC/MS and root exudate profiles were obtained. The analyses of the data allowed the correlation of characteristic chemical compounds and accessions. Several known chemical substances were found, but other compounds are still to be identified. We use MSMS methods to find not only the sum formula of those compounds but also to get information about their chemical structure. Using the MAGIC lines should provide a way to identify the genetic background of an interesting metabolic phenotype.

## Priming of resistance against Arabidopsis bacterial leaf speck by bacterial volatiles in Arabidopsis

P73

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Upon inoculation by avirulent pathogens or chemical agents, plants can develop an induction of systemic resistance (ISR) against further pathogen attacks. In this case, plant normally does not maintain but prime itself for potentiated defense machinery referred to as 'priming'. The priming has reported cost effective type of resistance to biotic and abiotic stresses. The role of priming in ISR is more ambiguous. In this work effect of volatile compounds of *Bacillus subtilis* GBO3 on priming of defence related genes including *PR1*, *PDF1.2*, and *chiB* were investigated at 0, 3, 6 and 12 hours after challenge of a semi-biotrophic pathogen *Pseudomonas syringae* pv. tomato DC3000. For testing priming state, results exhibited that bacterial volatiles emitted from strain GBO3 not only increased growth of plants but also decreased disease severity significantly. In genetic scale, bacterial volatile primed expression of *PR1* and *PDF1.2* but not *chiB*. According to these results our results indicate that bacteria volatiles prime salicylic acid and jasmomonic acid dependent signaling leading to protect plants effectively against potential pathogen attack.

## Belowground truffle ecology: spatial and seasonal variability in soil metabolites and fungal biomass within a truffle field

P74

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Truffles are symbiotic ectomycorrhizal fungi which develop on plant roots. Some truffle species (e.g. the Périgord truffle *Tuber melanosporum*) inhibit the development of non-host grasses in an area known as the 'burnt'. The mechanism behind this phenomenon is unknown and might involve (i) the production of phytotoxic compounds, (ii) competition for nutrients/ water, (iii) the direct attack on plant roots and, (iv) other type of indirect competition.

We tested the first two possibilities by collecting soil samples in a truffle field over 5 seasons (2x summer; 1x winter, 1x spring, 1x autumn). Soil samples were analyzed by an untargeted approach (metabolic profiling) and by targeted approaches (fungal biomass determination by ergosterol, analysis of phytohormones, nutrients and water content).

Neither metabolic profiling, nor nutrient analysis or water content resulted in significant differences among soil samples collected from inside or outside burnt zones. Phytohormone profiling, however, revealed that in all seasons except spring, jasmonic acid (JA) was significantly higher in samples collected outside burnt zones and fungal biomass followed a similar trend. Since JA is generally induced by plants mycorrhized by arbuscular mycorrhizas (AM), our results suggest that an indirect competition of truffles towards AM fungi might result in the 'burnt'.

## Comprehensive metabolite profiling of root exudates of *Arabidopsis thaliana*

P75

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Non-targeted metabolite profiling of root exudates of *Arabidopsis thaliana* cultivated in a hydroponic system has not been carried out so far. This is mainly reasoned by the lack of suitable experimental systems (sterile and high-throughput) and proper sample preparation protocols (enrichment of low-abundant metabolites and depletion of bulk compounds). Thus, we developed a new hydroponic system and extended the sample preparation workflow usually applied for metabolite profiling studies.

Briefly, we cultivated plants in a two-stage process and analysed root exudates from the fourth until the sixth week of plant cultivation while exchanging the medium every week. After sampling, cultivation medium was evaporated to dryness, reconstituted and subjected to two solid phase extraction cartridges (Reversed-Phase and Strong Anion Exchanger). Finally, both eluates were split and analyzed using two distinct analytical platforms (LC-MS and GCMS), which cover different parts of the metabolome.

Consequently, we could detect 120 compound spectra in UPLC-ESI-QTOF-MS profiles and 67 deconvolutions in GC-EI-QUAD-MS profiles in the course of the sixth week of plant cultivation. They comprise amino acids, sugars, polyols, hydroxylated organic acids, hydroxycinnamic acids, benzoic acid derivatives, nucleosides and sulfoxides.

## Functional metagenomics for evaluating biostimulation of native *quorum* - *quenching* populations in the potato rhizosphere

P76

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The phytopathogenic bacteria *Pectobacterium carotovorum* uses the N-acylhomoserine lactones (NAHL) signal molecules to regulate the expression of virulence genes. The inactivation of such signals therefore permits to reduce the aggressiveness of the pathogen, hence the symptoms. One antivirulence strategy consists in the application of gamma-caprolactone (GCL) to stimulate the growth of indigenous, NAHL-degrading bacteria in the rhizosphere of *Solanum tuberosum*. To evaluate the impact of GCL treatment on total bacterial populations, high throughput sequencing of amplified *rrs* was performed. However, a metagenomic library was constructed with total DNA extracted from GCL-treated rhizosphere and screened for the presence of fosmids conferring NAHL-degradation ability upon their *Escherichia coli* host. On one hand, structure of bacterial community showed a significant bias under GCL-treatment, especially an increase of NAHL-degrading *Rhodococcus* population. On the other hand, one clone, containing a 50 kb insert was thus identified by the screening of the library. One gene, *qsdB*, was identified as the gene responsible of the NAHL degradation. To conclude, GCL treatment induced a modulation of bacterial populations and metagenomics permitted identification of a novel gene responsible for NAHL degradation and its utilization as a potential molecular marker of GCL treatment.

## The effect of contrasting fertilization regimes on root gene expression and associated rhizosphere community composition

P77

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There is concern over the sustainability and environmental impact of mineral fertilizers used in intensive arable crop production systems. However, replacing mineral with organic fertilizers may lead to significantly reduced crop yield, as a result of lower levels of nutrients available to the plant. The aims of this research are to investigate the effects of fertilizer type and application rate on wheat root gene expression and rhizosphere community composition in four varieties of winter wheat (*Triticum aestivum*). Wheat varieties were grown in a pot experiment during May - August 2011 with five different N fertilization regimes (mineral NPK: 85 kg /ha and 170 kg N/ha; composted cattle manure: 170 kg N/ha and 340 kg N/ha; control: 0 kg N/ha). Roots in the upper 30cm of soil were sampled at the wheat flowering stage to analyse: 1) differential gene expression by reverse genetics, which combines transcriptomics (analysis of gene expression) and proteomics (analysis of products of gene products - proteins); 2) microbial rhizosphere community composition. Further root sampling took place at grain harvest to measure final root architecture. The transcriptomics and proteomics analysis is currently ongoing and is expected to enable the identification of genes that code for products involved in nutrient uptake. Complementary information from the analysis of microbial rhizosphere community composition will aid in the identification of expressed genes that are involved in root - microbial interactions, and how these interactions may contribute to differential nutrient uptake in contrasting fertilization regimes.

## Selection of plant growth promoting rhizobacteria by plant model species

P78

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Plant growth is dependent on many factors including the influence of soil bacteria. These influences may be negative, as with plant pathogens or positive, driven by plant growth promoting rhizobacteria (PGPRs). However, knowledge of how plants select for PGPRs in the rhizosphere is currently very limited. To address this, we selected rhizosphere bacteria over three generations of growth with *Arabidopsis thaliana*, *Medicago truncatula* and *Brachypodium distachyon*. Total rhizosphere microbial community was assessed by bacterial Automated Ribosomal Intergenic Spacer Analyses (ARISA) and by 454 pyrosequencing. ARISA revealed that the bacterial community differed significantly between plant rhizospheres and generations. 454 pyrosequencing confirmed this observation and allowed identification of the most dominant taxa. 16S rDNA sequencing of isolated bacteria was compared to the total community. This enabled identification of the rhizosphere-specific and independent species, belonging to *Achromobacter xylosoxidans* and *Arthrobacter oxidans* species, respectively. The strains identified here are ideal candidates to study the mechanisms of plant growth promotion in model plants rhizosphere. In the next step we will sequence genome of them and we will compare gene expression between laboratory cultures and rhizosphere cultures.

## Community metatranscriptomic analysis of wheat, oat and pea rhizospheres

P79

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Soil microbes are of global importance to nutrient cycling, and maintenance of plant health through interactions in the rhizosphere. Plant growth promoting rhizobacteria aid plants by providing nutrients, defending against pathogens and negating plant stresses. However current knowledge is limited to relatively few cultured representatives. We've used high-throughput sequencing of RNA to compare the active microbial communities in soil and the rhizospheres of wheat, oat and pea.

Total RNA was extracted from rhizosphere soil of 4 week old plants and unplanted controls. Samples were reverse transcribed, amplified, and sequenced using 454 technology. Ribosomal RNA (rRNA) sequences were identified using USEARCH and a small subunit rRNA database, then analysed in MEGAN.

Samples were dominated by Proteobacteria, Actinobacteria and Firmicutes. Wheat selected for Firmicutes, oat selected for Chloroflexi, and pea selected for Streptomyetaceae. Oat and pea selected for Chytridiomycetes and other fungi. Cellulose degrading bacteria were more abundant in wheat and oat rhizospheres, while members of the Verrucomicrobia, some methylotrophic bacteria, and nematodes, were more abundant in all rhizosphere samples.

Plants exerted subtle but specific effects on their rhizosphere communities. Microbes selected in the rhizosphere could have potential plant growth promoting properties. Efforts to culture and characterise representatives of these taxa should be improved.

## Bacterial volatiles suppress growth of fungal pathogens

P80

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Volatiles produced by soil bacteria did suppress fungal pathogens either by inhibiting growth or preventing spore germination. Results correlated with crop infection in bioassays

Bacterial volatiles might be components of soil suppressiveness. Previous studies (e.g. Chuankun et al. (2004); Zou et al. (2007)) showed differences in volatile profiles of soils with different degrees of fungistatic activity. In our experiments strong reduction of mycelial density of fungal pathogens by soil or extracted soil bacteria was found with a 2-side plate approach. Results from this experiment correlated with bioassays; increase in the number of disease symptoms and reduced root biomass was directly linked to reduced volatile suppression.

Future research is focusing on indentifying active compounds and the responsible microbes and genes. Furthermore, experiments will be set up to link volatile production to availability of organic resources to microbes e.g. sub fractions of soil organic matter. Understanding the relationships between plant pathogens, disease suppressive microbial populations, soil characteristics and bio-available substrates is essential to develop procedures for effective and consistent control.

## Functional analysis of the secretome of the vascular wilt pathogen *Verticillium dahliae*

P81

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Plant diseases cause severe crop losses worldwide. Vascular wilt pathogens are notoriously hard combat because they lack curative treatments and survive for decades in soil via persistent resting structures.

The soil-borne vascular pathogen *Verticillium dahliae* causes wilt disease on >200 plant species, including economically important crops and Arabidopsis. The proteins secreted by pathogens (secretomes) generally determine the outcome of host-pathogen interactions. Bioinformatic analyses of the recently released *V. dahliae* genome sequence predict over 780 secreted proteins. When excluding the cell wall-degrading enzymes, 460 secretome genes are identified encoding potential effectors that may potentially govern disease establishment. To identify proteins that modulate host immunity, a screen was developed in which secretome cDNAs would be constitutively expressed in Arabidopsis and analyzed for effects on plant defense (by screening for altered susceptibility towards various pathogens). This way, in an initial pilot screen with 45 single exon candidates, two effectors have been identified that affect host immunity. These two *V. dahliae* effectors will be subjected to further genetic and biochemical analyses to reveal their mode of action.

## Assessment of 16S rDNA hypervariable regions for screening bacterial diversity in complex soil environments with multimillion sequence read generating technologies

P82

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Multimillion read generating, high throughput sequencing technologies have become powerful with prospects in resolving 16S rDNA bacterial diversity of highly complex soil environments. However, contemporarily these technologies are length-wise restricted in screening DNA stretches of single 16S rDNA hypervariable (V) regions. Aim of the present study was to assess effects of properties of four consecutive V regions (V3-6) on commonly applied analytical methodologies in bacterial ecology studies. This was carried out by exploiting commonly used non-redundant 16S rDNA sequence databases and also simulated samples according to previous high throughput sequencing studies. Results indicate that primers targeting the mere 16S rDNA gene should be *de novo* designed for soil environments, since previous 16S rDNA conservation studies were based on databases heavily dominated by the less diverse human microbiome sequences with database participation of up to 54 %. V3 and V4 showed an overall higher representation of the full-length 16S rDNA variants with V4 however lacking highly conserved flanking sites that would allow primer designing for maximum diversity screening. V6 had the poorest performance throughout all analyses. Based on these results, theoretical assessment of the experimental approach is strongly suggested prior performance of 16S rDNA based bacterial diversity studies.

## Bacteria associated with ectomycorrhizal roots of the arctic-alpine plant *Bistorta vivipara*

P83

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The mycorrhizal symbiosis between fungi and plant roots are among the most ancient and prevalent on Earth. This symbiosis has been crucial for the assembly, functioning, evolution and current composition of terrestrial ecosystems as it mediates nutrient acquisition in the majority of land plants. However, the intimate partnership between plant and fungi may also be influenced by a possible third component of mycorrhizal symbiosis, bacteria. These could be loosely or tightly associated with mycorrhizal fungi and most likely play a role in mycorrhizal functioning. Increasing evidence shows that bacteria-fungi interactions are more widespread than expected and that their dynamics may be important in the ectomycorrhizal symbiosis. Here, both fungi and bacteria occurring in root systems of a local population of *Bistorta vivipara* were analyzed using high-throughput pyrosequencing of 16S (bacteria) and ITS rDNA (fungi) amplicons. We explore (a) the bacterial diversity associated with the Ectomycorrhizal root-systems, and (b) analyze potential relationships between fungal and bacterial diversity.

## Tree triangles, nitrogen and microbial communities: who has the upper hand on ecosystem processes?

P84

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Microorganisms regulate central ecosystem processes, but it is unclear how they respond to biodiversity change and nitrogen (N) deposition. We evaluated the effects of plant diversity and N addition on microbial communities in an unpolluted temperate forest in Patagonia, identifying monospecific microsites dominated by a single *Nothofagus* tree species, and plurispecific microsites dominated by three *Nothofagus* species, with and without an N treatment (0 and 80 kg/ha/yr). Previously, we demonstrated that plant species and N addition affected microbial functioning, with a home-field advantage for litter decomposition in its origin, and strong N stimulation of litter decomposition. We pyrosequenced bacterial 16S rRNA genes and fungal ITS region to analyze microbial communities in the forest floor. Plant species affected the composition of bacterial and fungal communities even at the level of phylum; in contrast plant diversity had no effects on microbial composition. N addition consistently reduced bacterial and fungal richness, and this reduction was dependent on the identity of the plant species. Our results suggest that long-term plant-soil feedbacks can modulate the composition of microbial communities, and could explain the observed home-field advantage for litter decomposition. In contrast, disruption of these interactions with N addition altered microbial communities with important consequences on ecosystem functioning.

## A forward genetic *in planta* screen to identify plant-protective traits of *Sphingomonas* sp. Fr1 against *Pseudomonas syringae*

P85

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Members of the genus *Sphingomonas* belong to the predominant phyllosphere colonizers of several plant species. Plant-indigenous *Sphingomonas* have been shown to protect *Arabidopsis thaliana* against the bacterial leaf pathogen *Pseudomonas syringae* DC3000. We have developed a forward genetic *in planta* screen to identify genes of the beneficial *Sphingomonas* sp. Fr1 necessary for this effect. About 5000 mini-Tn5 mutants of *Sphingomonas* sp. Fr1 were assayed for a defect in plant protection against a *luxCDABE*-tagged *P. syringae* DC3000 derivative in a space-saving 24-well plate system. Bioluminescence of the pathogen was used as readout for pathogen colonization and allowed the identification of *Sphingomonas* sp. Fr1 mutants that had lost the ability to restrict pathogen growth. Of these mutants, eleven were validated as plant protection defective, yet colonization competent. The mutants were evaluated in a previously described standard microbox system and plants were confirmed to show enhanced disease phenotypes relative to those inoculated with the parental strain as control. In conclusion, the established screening protocol allowed us to identify mutants and opens the possibility to uncover traits important for *in planta* microbe-microbe interactions.

## Characterization of an AM-responsive MYB-like transcription factor during the symbiosis between *Lotus japonicus* and arbuscular mycorrhiza fungi

P86

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The interactions between legumes and arbuscular mycorrhiza (AM) fungi are vital to the development of sustainable plant production system. So far little attention has been given to the role of transcription factors (TFs) in AM symbiosis establishment and development. Transcriptomic analysis performed on *Lotus japonicus* showed that the second highest upregulated gene was a MYB-like TF (*LjMYB*) and that the transcripts were localized into arbusculated cortical cells.

Phylogenetic analysis showed a strong similarity between the *LjMYB* sequence and the sequences of plant proteins involved in P starvation response. To confirm a role of *LjMYB* as TF, the p35S::eGFP::LjMYB construct revealed a nuclear localization in *Arabidopsis* protoplasts, while in transgenic *Lotus* plants expressing the GFP-*LjMYB* fusion protein, the fluorescent signal was exclusively located in the nuclei of the arbusculated cells, but disappeared with the arbuscule collapse. GUS transformed plants revealed a staining not only in the arbusculated cells, but also in the apex of roots, irrespectively of the fungal presence. An approach of reverse genetics was performed to understand the biological and molecular functions of *LjMYB*. Taken in the whole, the results support the involvement of the AM-responsive *LjMYB* in crucial plant physiological and developmental processes.

## Bring the field to the lab - why 'antimicrobial' plants should be tested with native bacteria

P87

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The wild tobacco *Nicotiana attenuata* has been used in recent years as a model plant in ecological research. To reveal the hidden influence of non-culturable bacteria in the field we constructed stable transformed plant lines by ectopic over expression of small antimicrobial peptides.

In leaf inoculation experiments *in vivo* activity of the transgenic plants could be shown with the growth promoting bacterium *Bacillus pumilus* DSM 1794 but not if the pathogen *Pseudomonas syringae* pv. *tomato* DC 3000 is used in the same experiment. Since we are interested in effects on the native bacterial community we extended the inoculation with bacteria, previously isolated from roots and seeds of *N. attenuata*. Most of the isolates including also a 'wild' *Bacillus pumilus* strain do not show a reduction of CFU after inoculation. Preliminary field data also indicate no growth or fitness impairment of the transgenic plants if grown in the native environment.

This points out the insufficiency of antibiotic activity data if they derive from *in vitro* studies only. We propose the *in vivo* use of native isolates for a more realistic view about the activity under natural conditions and underline the importance of field experiments for the exploration of ecological relevant interactions.

## Genetic variability of the Verticillium syndrome in an Arabidopsis mapping population

P88

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Verticillium species colonise the plant root surface in response to root exudates, penetrate the cortex and endodermis, and spread systemically through conidia transported by the transpiration stream in the xylem. Recent studies made use of the model plant *Arabidopsis thaliana* to elucidate the genetic fundamentals of the Verticillium pathosystem as available accessions cover a wide range of responses towards infection. Here we report on the application of the Arabidopsis MAGIC (Multiparent Advanced Generation Inter-Cross) population to study genotype-specific responses of the Verticillium syndrome. All nineteen parent lines of this population were tested for their reaction in the pathosystem with *V. dahliae* to identify lines revealing a strong phenotype upon interaction. Two lines were identified with contrasting phenotype and fungal colonisation, that is Bur-0 was tolerant while Ler-0 was sensitive towards the pathogen.

Arabidopsis and other Brassicaceae produce glucosinolates, secondary compounds with biocidal properties. The hydrolysis products of glucosinolates are formed upon tissue disruption and are known to suppress fungal growth. In order to determine the effects of volatile composition on pathogen growth, we have characterized the glucosinolate patterns of all parent lines and tested the biological activity of released glucosinolates in biofumigation assays. Results of these analyses are presented here.

## The microbial ecology of plant-soil feedback: exploring the relationship between microbial plant preference and feedback potential

P89

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Plant-soil feedback refers to a general suite of interactions in which soil microorganisms respond to plant presence and, in turn, affect the fitness of the plant for good or ill. A microbe-centered approach to plant-soil feedback has potential to uncover heretofore unknown relationships between plants and soil microorganisms. Using empirical data from a series of ‘home’ vs. ‘away’ experiments involving common sunflower and giant ragweed, we characterized over 600 bacterial and fungal taxa as individual agents of feedback. We used Redundancy Analysis to score each microbial taxon according to an index of preference for ragweed or sunflower, and we used Partial Least Squares Regression to quantify the positive or negative feedback potential of each microbial taxon to the growth of the plants. We found significant ( $p < 0.001$ ) linear relationships between microbial preference and feedback potential in 75% of cases. Microbes with a high preference for ragweed also reduced ragweed biomass (i.e. negative feedback potential), while those with a high preference for sunflower increased ragweed biomass. Microbial feedback to sunflower was more variable. Thus, ragweed appears to generally attract microbes with negative feedback potential, while sunflower may attract either positive or negative microbes, depending on the particular species pool.

## Using PacBio RS reads to improve microbial genome assemblies

P90

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Advances in genome sequencing are accelerating bacterial genomics. However, building finished assemblies of microbial genomes remains a tough challenge. The most enticing attribute of third-generation sequencing technologies, such as SMRT sequencing from Pacific Biosciences, is long read lengths. Unfortunately, *de novo* assembly of genomes with PacBio RS reads has proven difficult because of high error rates primarily consisting of insertions and deletions. An emerging hybrid assembly technique utilizes high quality reads from second-generation sequencing platforms such as Illumina to correct long error-prone PacBio reads. With error-corrected PacBio reads, long read assemblers such as the Celera Assembler, can be used for *de novo* assembly. An alternative hybrid assembly technique first builds a draft genome using second-generation sequencing technologies and subsequently links adjacent contigs spanned by long PacBio reads. Using the DC3000 strain of *Pseudomonas syringae* pv *tomato*, we investigate assembly strategies with combinations of PacBio reads, Illumina reads, and already existing draft genomes with a primary focus of building finished assemblies of microbial genomes. Our data suggest that third-generation sequencing technology can help assemble better genomes, but that significant changes in how we assemble these genomes are required.

## Metagenomic analysis of bacterial endophytic communities associated with grapevine (*Vitis vinifera* L.)

P91

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In recent years, interest in endophytic microorganisms has increased, as they play a key role in agricultural environments and are promising because of their potential use in sustainable agriculture. These microorganisms include both commensal species, which have no direct effect on the host plant, and mutualistic symbionts, which could be used in the biological control of pathogens or plant growth promotion. In the present study we investigated how microbial communities in plants from organically managed farms differ from those obtained from integrated pest management (IPM) farms. Microbial DNA isolated from grapevines (*Vitis vinifera* L.) cv Merlot and Chardonnay cultivated in a subalpine area in Northern Italy was PCR amplified to fingerprint endophytic communities, and to assess the distribution of important functional genes in the grapevine microbiome in the studied areas.

Here we report the composition of endophytic microbial communities assessed through a cultivation independent approach: Automated Ribosomal Intergenic Spacer Analysis (ARISA). The changes in community structure and composition are interpreted in the light of the environmental variables considered. Fingerprinting results were validated by multivariate analysis. Other metagenomics approaches are being considered.

## Mating type gene search and quantification of *Tuber melanosporum* as tools to evaluate truffle-ground productivity

P92

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Among the microbial communities of plant microbiome, mycorrhizal fungi occupy a crucial position. The genome sequencing of the ectomycorrhizal fungus *Tuber melanosporum* has revealed that the fruiting body production depends on the availability in the soil of two mycelia with opposite mating types. This finding has suggested that seedlings produced for truffle-culture programs have to be inoculated with both the mating types. In order to investigate if the productivity is also correlated to a certain amount of *T. melanosporum* in the soil, in addition to the co-presence of mating type genes, we set up a protocol in a model truffle-ground presenting productive and unproductive trees. The quantity of *T. melanosporum* in soil samples was assessed by qPCR on ITS region and its mating types were searched. Results showed that mating type genes were detected in the stand under productive trees when more than 0.3 ng of *T. melanosporum* DNA was present. Up to now the establishment of a *T. melanosporum* plantation has been exclusively based on soil features. Nowadays the proposed analyses can help truffle operators in the management of their plantation by attesting the occurrence of *T. melanosporum*, after seedling inoculation and before the harvest of the fruiting bodies.

## ***Lotus japonicus*-a model species for root-microbiome interaction studies**

**P93**

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Legumes have the capacity to recognise and benefit from a broad spectrum of microbe associations establishing root nodule symbiosis (RNS), arbuscular mycorrhiza (AM) symbiosis, and endophytic association. Furthermore, besides these beneficial associations, legumes are also hosts for various pathogenic organisms, therefore providing a valuable system for studying multiple responses within an individual host plant. The existence of a common genetic program for RNS and AM was demonstrated. Furthermore, corresponding components from monocots can perform the same function in legumes, demonstrating the establishment of this genetic program before the divergence of monocots and dicots. The recently demonstrated ability of *Lotus* spontaneous nodule cells to be colonized, and to be intracellularly infected by Nod-factor defective rhizobia indicate that genetic components of an ancient infection/colonization program still operate in *Lotus* nodules. We are taking advantage of the large panel of resources available for *Lotus japonicus* to study the endophytic root colonization. Our results show that this model legume is colonized by *Azospirillum brasiliense*, a broad host-range endophyte of monocots, and that *Lotus* roots mount a genotype-dependent response. Furthermore, we have isolated *Lotus* native endophytes, whose colonization pattern and the induced response will be presented.

## **Plant-endophyte-pathogen interactions in poplar**

**P94**

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Fungal endophytes are ubiquitous within the leaves of all plants surveyed to date, yet very little is known about their functional roles. Previous studies have shown the potential for endophytes to reduce the damage to plants from foliar pathogens. Yet, few have examined the complex set of interactions that occur between host plant genotype, fungal endophytes, and plant pathogens. We sought to quantify these interactions in a fully factorial manipulative greenhouse experiment focusing on six different genotypes of *Populus angustifolia*, two endophytes, and the fungal necrotrophic pathogen *Drepanopeziza populi*. Counter to our expectations that both endophytes would confer resistance to the pathogen, one nearly doubled its host plant's susceptibility (in terms of premature leaf abscission and leaf area damaged compared to a control, Tukey HSD  $p < 0.001$ ). To understand potential mechanisms for this effect, we also assessed via QPCR a range of gene transcripts related to plant defense. We found that the same endophyte that resulted in increased pathogen susceptibility caused significant down-regulation of the defense-related protein PtATOSM34 following pathogen inoculation. These results may thus point to the potential for certain species of endophyte to exacerbate damage by fungal plant pathogens, with implications for management and forest health.

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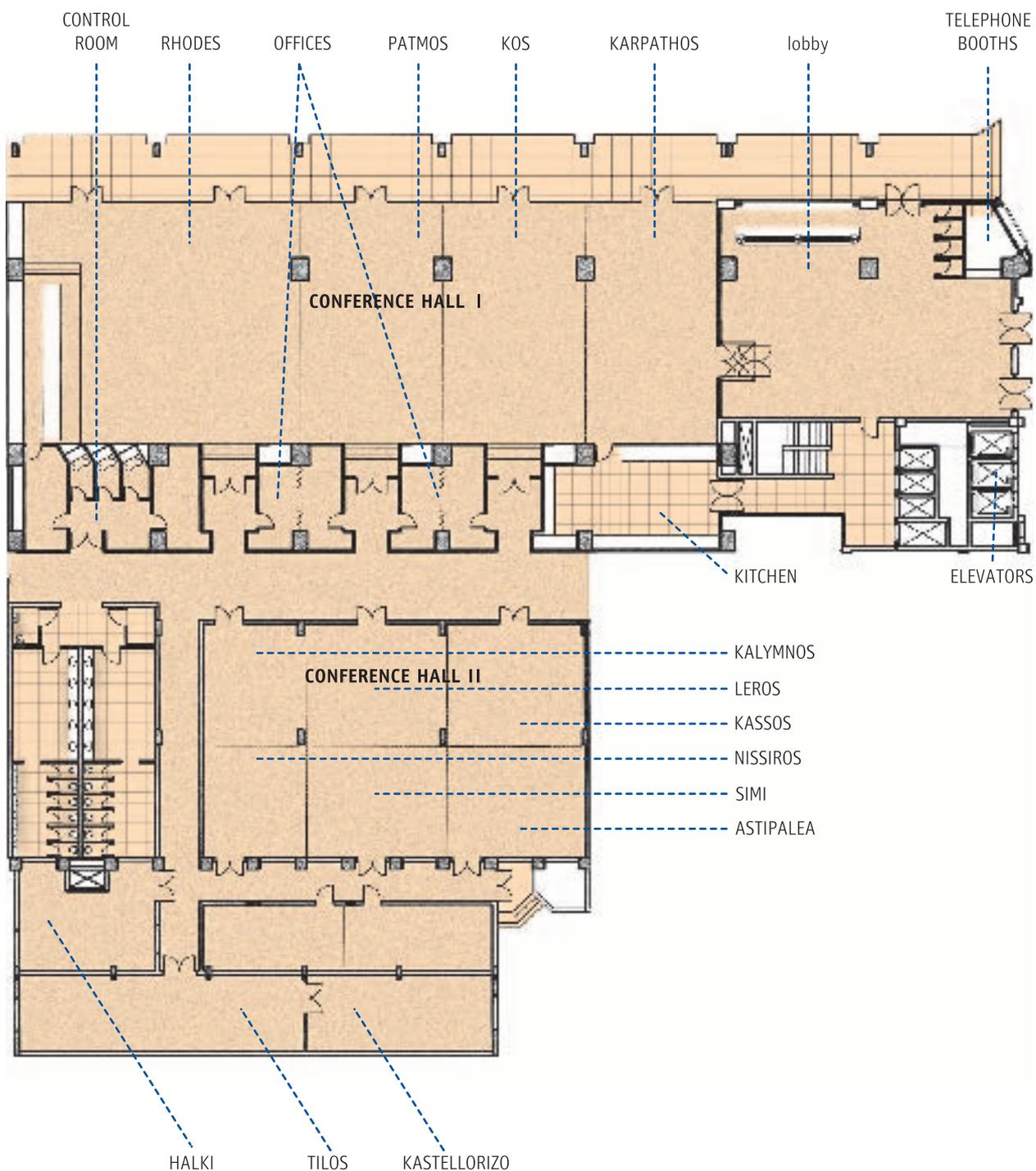
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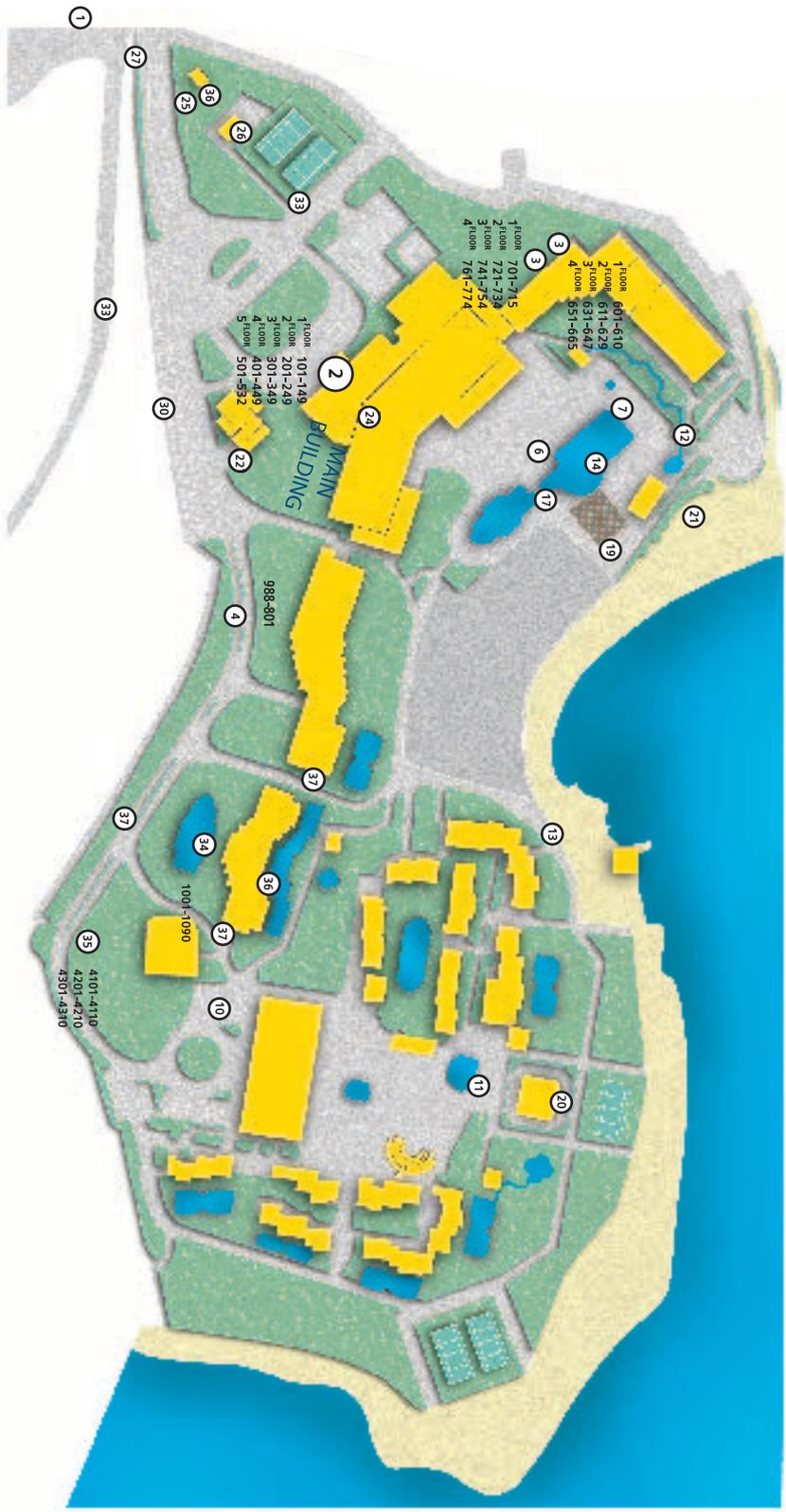
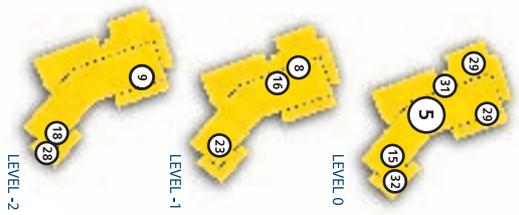
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# C O N F E R E N C E C E N T R E



**COLLOSSOS  
MAIN BUILDING**



- GENERAL**
- 1 MAIN ENTRANCE
  - 2 MAIN BUILDING "COLLOSSOS"
  - 3 BUILDING "NEFFEL"
  - 4 BUILDING "DIACORAS"
  - 5 RECEPTION PARADISE MARE
  - 6 MAIN POOL
  - 7 WATERSLIDE

- RESTAURANTS & BARS**
- 8 MAIN RESTAURANT "SYMPOSIO"
  - 9 SELF SERVICE / SMACK BAR & ITALIAN RESTAURANT "LA PERGOLA"
  - 10 CHINESE RESTAURANT
  - 11 BEACH RESTAURANT & GREEK TAVERN "THALASSA"
  - 12 BEACH RESTAURANT / SELF SERVICE "ALBATROS"
  - 13 BEACH BAR "NAUTILUS"
  - 14 FRENCH A LA CARTE RESTAURANT "DIONYSOS"
  - 15 MAIN BAR "CHEVALLER"
  - 16 COCKTAIL BAR "EVA"
  - 17 POOL BAR "DELFINIA"
  - 18 NIGHT CLUB "SAFARI"

- SPORTS & RECREATION FACILITIES**
- 19 BEACH
  - 20 BEACH VOLLEY
  - 21 WATERSPORT CENTER
  - 22 MINI GOLF
  - 23 INDOOR POOL / PLAY ROOM
  - 24 ANIMATION STAGE / OPEN AIR THEATER
  - 25 MINI CLUB
  - 26 TENNIS COURTS

- VARIOUS**
- 27 CHURCH
  - 28 CONFURE FITNESS CENTRE, SAUNA, HAMMAM, JACUZZI
  - 29 CONFERENCE CENTER
  - 30 MINI MARKET
  - 31 SHOPPING ARCADE, JEWELLERY, KIOSK, BOUTIQUE
  - 32 BRIDGE ROOM / TV - DVD ROOM
  - 33 PARKING
  - 34 BUILDING "KAMIROs"
  - 35 BUILDING "KASTILO"
  - 36 CHILDREN'S POOL
  - 37 POOL

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PARADISE MARE  
ALL INCLUSIVE