Questions: Are proteins with virulence factors involved in the symbiotic interaction of two partner bacteria? Is it possible to assess a metabolic coupling between the two partners?

Methods: In order to elucidate the role of the corresponding proteins in the symbiotic interaction between the two partner bacteria, recombinant proteins were expressed in E. coli and used to produce antibodies for immunogold labelling and high resolution fluorescence microscopy for intracellular localization. The possibility of metabolic coupling between the two partner bacteria was assessed by nanoSIMS, magnetic capture and transcriptome analyses.

Results: The proteins encoded by the three symbiosis genes are transported across the cell envelope of the epibiont into the central bacterium. Cag 1919 contains a RTX domain which is typically found in Gram-negative pathogenic bacteria and was localized in condensed areas of the cell wall of the central bacterium. Cag_0614 and Cag_0616 represent the largest open reading frames (110418 and 61938 bp) known among prokaryotes and are transported across the cell envelope of the epibiont and are localized in the cytoplasm of the central bacterium. The epibiont provides metabolites to the central partner bacterium depending on the external nitrogen source and exogenous carbon substrates. The central bacterium, though not phototrophic, remains metabolically inactive in the dark and transcribed a variety of transporters.

Conclusions: The unexpected transfer of virulence factor-like proteins strongly suggests an involvement in the tight symbiotic interaction. There is also a metabolic coupling between the two partner bacteria which depends on the physiological activity of the epibiont.

(1) Vogl K et al. (2008) Environ. Microbiol. 10:2842-2856.

MCV16

Global and local patterns of bacterial communities associated with peatland bryophytes

*A. Kiss¹, A. T. Tveit², M. Winkel¹, F. Horn¹, T. Hájek³, M. M. Svenning², D. Wagner¹, S. Liebner¹ ¹GFZ German Centre for Geosciences, Helmholtz Centre Potsdam, Section 4.5 Geomicrobiology, Potsdam, Germany ²UiT The Arctic University of Norway, Department of Arctic and Marine Biology, Tromsø, Norway ³University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic

Introduction: Northern peatlands are pristine ecosystems that act as important global carbon stocks in which C- and N-cycles are driven by plants (mosses, sedges) and a specialized microbiota. Bryophytes like Sphagnum and Amblystegiaceae ('brown mosses') are typical representatives of these peatlands. Recent studies focusing on Sphagnum mosses and other bog plants identified an essential core microbiom¹, but also uncovered Sphagnum-species specific bacterial groups². A comprehensive sampling design is lacking which includes brown mosses and aims at elucidating the influence of environmental parameters shaping the moss-associated bacterial (MAB) community at a global scale.

Questions: We aimed at describing the influence of environmental parameters on the MAB community at the local and the global scale.

Methods: In a systematic approach, we sampled mosses and references (sedges and/or sediment) from 26 sites at 4 different locations: Svalbard (High Arctic, Spitsbergen), Samoylov (Arctic, Siberia), Neiden (Subarctic, Norway) and Mueritz (Temperate, Northeast Germany). After separation of endo- and epiphytic bacteria of MAB, Illumina sequencing of the 16S rRNA gene was applied. Cell wall analysis (lignin-like polymers, holocellulose, CEC) and C/N determination of mosses and sedges were carried out. Environmental parameters (pH regime, DOC, ions, organic acids) as well as temperature, CH4 and O2 gradients were measured and used for statistical analysis.

Results: The sample sites represent two different ecosystem types: neutral pH peatlands with submerged brown mosses (Svalbard, Samoylov) and low pH peatlands with emerged/submerged Sphagnum mosses (Neiden, Mueritz). These types were also reflected in two distinct global clusters of the MAB communities. The low pH clusters generated different MAB subclusters of emerged and submerged Sphagnum-associated species and additionally displayed differences on the local scale. Overall, the brown moss sites showed a higher diversity than the Sphagnum sites. A more defined analysis on the endophytic MAB showed a high dominance of Alphaproteobacteria in the low pH Sphagnum sites in comparison to a high dominance of Actinobacteria in the neutral pH brown moss sites.

Conclusions: MAB patterns on the global scale reflect environmental controls driven by pH and hydrology. Within each studied site, highly similar, but distinct MAB communities occupy local environmental niches represented by subsites. Additionally, we identified specific, siteindependent, endophytic MAB in Sphagnum and brown mosses.

- 1. Bragina, A., Berg, C. & Berg, G. The core microbiome bonds the Alpine bog vegetation to a
- Dragind, A., Berg, C., & Berg, O. The core microtonic bonds the Alpine bog vegetation to transkingdom metacommunity. *Mol. Ecol.* 24, 4795-4807 (2015).
 Bragina, A., Berg, C., Müller, H., Moser, D. & Berg, G. Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. *Sci. Rep.* 3, (2013).

MCV17

Microbial hub taxa link host and abiotic factors to plant microbiome variation

*M. Agler¹, J. Ruhe¹, S. Kroll¹, E. Kemen¹

¹Max Planck Institute for Plant Breeding Research, Plant-Microbe Interactions, Cologne, Germany

Question: Microbiomes critically affect the physiology and performance of plant hosts and are thus subject to selection along with the host genome. Thus, evolution and ecology of plants can only be understood in a holobiont context that includes microbes that colonize the phyllosphere. A variety of factors influence host microbial community structures, but little is known about how these factors act on the microbial community and especially what role microbe-microbe interaction dynamics play.

Methods: We addressed this knowledge gap by simultaneously studying three major groups of Arabidopsis thaliana symbionts (bacteria, fungi, and oomycetes) using a custom amplicon sequencing protocol and a systems biology approach to analysis. We combined sampling of wild A. thaliana populations and field plantings to evaluate effects of location, sampling time and host genotype and isolated phyllosphere microbes to confirm findings in the lab with successive host colonization experiments.

Results: First, we confirmed previous results showing that abiotic factors and host genotype affect plant colonization. We then uncovered a network on inter-kingdom microbe-microbe interactions that affect community structure. A few taxa, which we term microbial 'hubs' were especially interactive in the phyllosphere. In-planta lab experiments supported field observations and proved an important mechanism whereby host and abiotic factors control colonization by hubs, which in turn transmit effects to the microbial community, resulting in observed correlations. Importantly, in our experiments with hub microbes including the obligate biotrophic oomycete pathogen Albugo, the basidiomycete yeast fungus Dioszegia, and the bacteria Caulobacter, we found evidence for direct interaction between the hubs in addition to direct effects on the larger microbial communities. Specifically, while Albugo generally caused decreased bacterial alpha diversity and stabilized beta diversity compared to uninfected plants, Dioszegia inhibited Caulobacter growth and promoted Albugo virulence.

Conclusion: Our results demonstrate the importance of hubs in host microbiome structuring and in turn the importance of the microbiome on hub fitness. Thus, both plants and microbial hubs are organisms with hologenomes and we hypothesize that the microbial community represents a location of indirect interaction and competition between host and these influential microbes. Thus, the identification of microbial 'hubs' has crucial implications for plant-pathogen and microbe-microbe research and opens new entry points for ecosystem management and future targeted biocontrol.

MCV18

Transcriptional profiling during in planta development of the corn smut fungus

*D. Lanver¹, A. Müller¹, N. Ludwig¹, G. Schweizer¹, F. Haas², A. Jarosz³, J. Altmüller³, S. Rensing², R. Kahmann¹

¹Max Planck Institute for Terrestrial Microbiology, Marburg, Germany ²Philipps University, Marburg, Germany

³Cologne Center for Genomics (CCG), Cologne, Germany

Ustilago maydis is the causative agent of corn smut disease. After penetrating the plant epidermis, the fungus grows as branching mycelium and induces tumors in colonized tissues. The fungal hyphae grow initially intracellularly but at later stages, i.e. during tumor formation, they form huge aggregates in apoplastic caves. Within these aggregates maturation of teliospores takes place. The transcriptional changes during the in planta development of this strictly biotrophic fungus are not well documented.

We performed RNAseq based transcriptional profiling of Ustilago maydis during seedling infection. The analyzed developmental stages range from plant penetration to induction of tumors and spore maturation. More than half of the 6700 Ustilago maydis genes are differentially expressed throughout the life cycle. The most highly expressed genes encode secreted effector proteins, which are needed to establish the biotrophic interaction between fungus and host plant. We categorized the 320