# Linking the microbiome to plant metabotype and high-value phytochemicals

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## Introduction

Plants form close interactions with microorganisms that are essential for their performance and survival. Thus, plant-microbe interactions are key for understanding and improving plant health and productivity and for sustainable agricultural management practices. It is well-known that plants use metabolites to direct organization and growth of their associated microbial communities. However, *vice versa*, the plant-associated microbiome influences the metabolic activity of the plant leading to different metabotypes. A significant number of plant metabolites are produced by associated microbes, or through interaction with their plant host (prominent e.g. paclitaxel). To analyze microbiome-metabolome interactions, we used the grass model *Brachypodium distachyon* as well as different species of medicinal plants with particularly high levels of complex constituents, including *Matricaria chamomilla* and *Calendula officinalis*. These two medicinal plants are cultivated all over the world, however with different chemical profiles. We observed a plant-specific selection of rhizospheric microbes associated with medicinal plants grown on an organically managed Egyptian desert farm. The soil microbiome comprised a high abundance of spore-forming *Firmicutes* (esp. *Bacillus* and *Paenibacillus*) and *Actinobacteria* (*Streptomyces*), which were linked to pathogen suppression under arid soil conditions. The desert agro-ecosystem exhibited a higher microbial diversity and better ecosystem function for plant health in comparison to the native desert soil. Promising antagonistic counterparts to soil-borne phytopathogens were selected by a hierarchical screening for field evaluation. The priming of chamomile seedlings had a stabilizing effect on plant performance, and indigenous *Bacillus* and *Paenibacillus* strains were also able to elevate the plants' flavonoid production. These findings suggest that a targeted bacterial treatment can influence the metabolic activity of the plant. We aim to reveal the underlying mode of actions at the genomic and transcriptional levels and to develop an effective biocontrol strategy on the basis of these promising antagonists.

## How are the native microbial communities influenced by 30 years of organic agriculture?

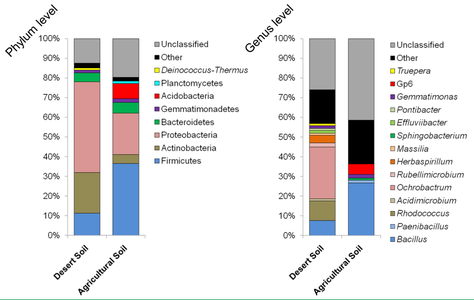



**Figure 1. The bacterial communities in the two different soil types.** Relative clone composition of major phyla and genera was determined by pyrosequencing of 16S rRNA from metagenomic DNA extracted from desert and agricultural soil. Phylogenetic groups accounting for ≤1% of all quality sequences are summarized in the artificial group others.

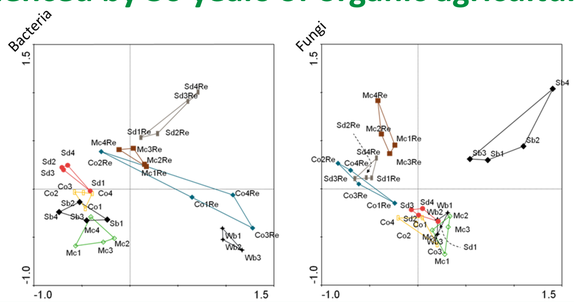



**Figure 2. Principal component analysis of OTUs identified by SSCP fingerprinting for bacterial and fungal communities.** Samples were encoded using a combination of letters and numbers indicating (1) soil type or plant species (Wb = desert soil, Sb = Sekem soil, Mc = *Matricaria chamomilla*, Co = *Calendula officinalis*, Sd = *Solanum distichum*), (2) replicate (1–4) and (3) microenvironment (Re = endorhiza, rhizosphere and soil have no further designation).

## Is the microbial community on a functional level as specific as on the structural level?

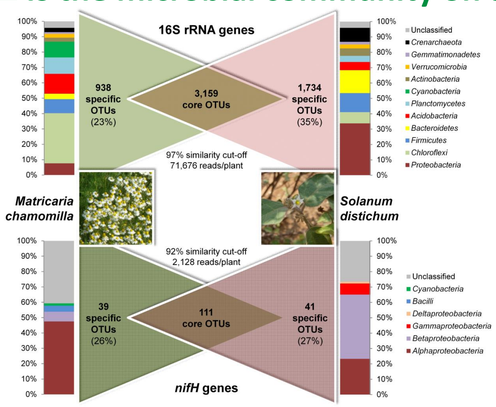



**Figure 3. Taxonomic composition and Venn diagrams of the 16S rRNA and *nifH* gene communities inhabiting the rhizosphere of *M. chamomilla* and *S. distichum*.** Both plants were cultivated in direct proximity to each other under field conditions (loamy sand soil) and were investigated in four independent replicate samples by amplicon sequencing. Singletons, OTUs defined by only a single observation, were removed and not considered in both datasets.

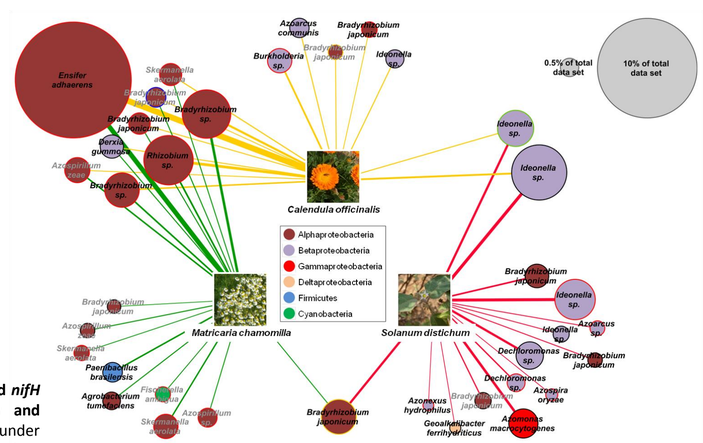



**Figure 4. Profile clustering network analysis of NifH sequence libraries of rhizosphere samples from *M. chamomilla*, *C. officinalis* and *S. distichum* at a dissimilarity level of 8%.** The abundance values for OTUs with a mean read change between plants of more than 1% of the normalized data set were used. If the ratio of mean OTU read numbers exceeded 2, the OTUs were regarded as altered and assigned to the respective profile. Node sizes of OTUs correspond to the relative abundance of the total data set; nodes matching to abundances of 0.5% and 10% were added as reference points. Distributions between plants are displayed by widths of connection lines. Significances ($p < 0.05$) are indicated by colored node borders: red node borders indicate significances between connected and all not linked profiles, green is used for significances between *Matricaria* and *Calendula*, orange for significances between *Calendula* and *Solanum*, and blue for significances between *Matricaria* and *Solanum*; nodes with black borders showing no significant differences. Black node labels indicate a similarity to the taxonomic node label (closest database match) of ≥95%, whereas gray node labels have a similarity <95%.

## Medicinal plants harbor a promising indigenous potential for promotion of plant growth and quality

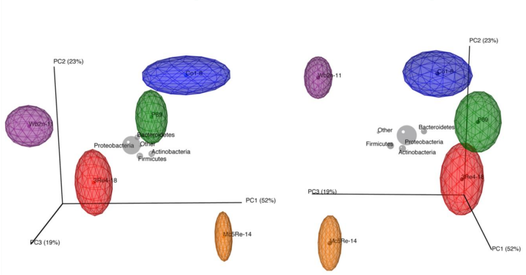



**Figure 5. Comparison of the microbial communities of *Matricaria chamomilla* rhizosphere by jackknifed principal coordinate analysis.** The biplot illustrates the compositional similarity between samples based on weighted UniFrac. Taxa coordinates are indicated by grey orbs with size, as a function of relative abundance. To confine the biplot, the number of the displayed taxa was restricted to 5. The positions of the points are the averages for the jackknifed replicates generated by QIIME and are shown with ellipses representing the interquartile range (IQR) in each axis.

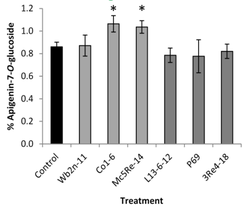



**Figure 6. Content (%) of apigenin-7-*O*-glucoside in *Matricaria chamomilla* samples.** Averages of individual HPLC-MS measurements and confidences are shown.

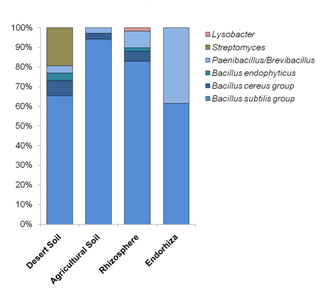
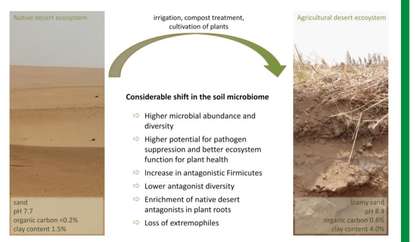



**Figure 7. Diversity of bacterial antagonists with an activity towards pathogenic fungi.** Isolates with activity against two pathogens were identified by partial 16S rRNA gene sequencing. Samples from rhizosphere and endorhiza include isolates from the medicinal plants *M. chamomilla*, *C. officinalis* and *S. distichum*.


## Conclusions




**The microbiome of medicinal plants grown on a desert farm under organic management**

- Antagonists under arid conditions are mainly Gram-positive
- Targeted bacterial treatments could influence the metabolic activity of the plant
- Broad diversity and high abundance of diazotrophs underline their importance for native and agricultural desert ecosystems
- Diazotrophic rhizobacteria of *M. chamomilla* and *C. officinalis* are dominated by potential root nodule bacteria
- The rhizosphere of *S. distichum* is higher colonised by free living nitrogen fixers
- Medicinal plant-specificity on a structural as well as functional level

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