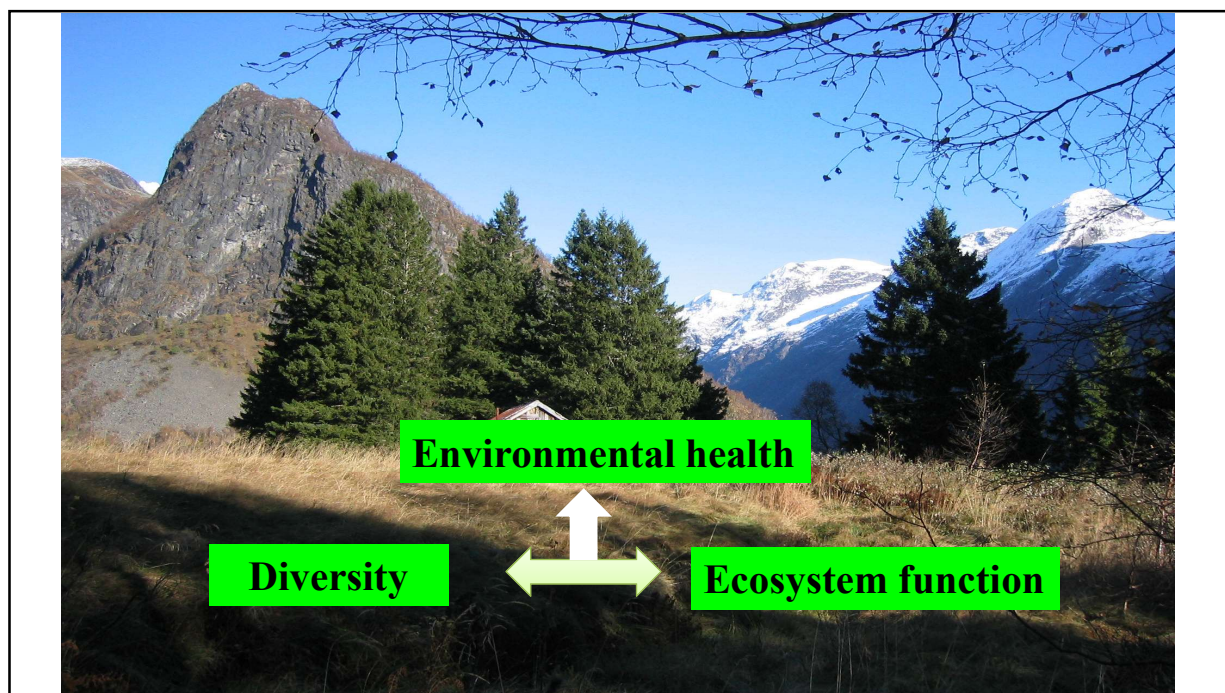


# Bacterial diversity and community composition analysis

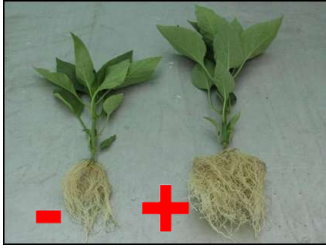
Silke Ruppel

Leibniz Institute of Vegetable and  
Ornamental Crops Großbeeren, Germany

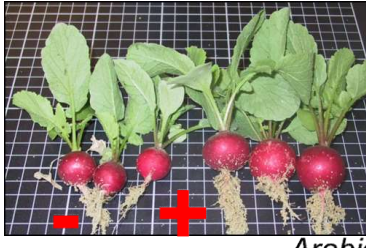


**Plant growth improvement after PGP bacterial application**


*Capsicum annuum*



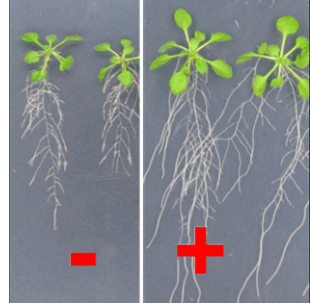
*Raphanus sativus var. sativus*

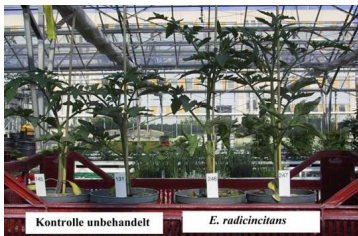


*Solanum lycopersicum*



*Arabidopsis thaliana, Col-0*



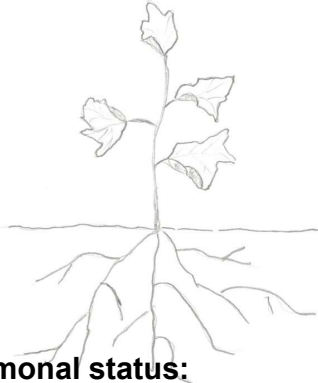


Kontrolle unbehandelt      *E. radicinians*

**Modes of Growth-Promotion by Endophytic Bacteria**

**Plant nutritional status:**

- Biological nitrogen fixation
- Ca-, Al- Fe-phosphate solubilization by bacterial carboxylic acid production
- Iron chelating



**Plant health status:**

- Bacterial production of antibiotics, vitamins, hormones
- Induction of plant systemic resistance
- Suppression of pathogens by enhanced colonisation rate

**Plant hormonal status:**

- Decrease in plants ethylene level via ACC deaminase
- Bacterial production of Auxine, Cytokinin, Gibberelline

If a single bacterial strain can improve plant growth so efficiently, which effect has the total plant inhabiting microbial community ?

Two examples:

- The phyllosphere bacterial community of raw digested herbs or vegetables inhabit about  $10^7$  bacterial cells per gram leaf with a high phylogenetic diversity



Cress



Thyme



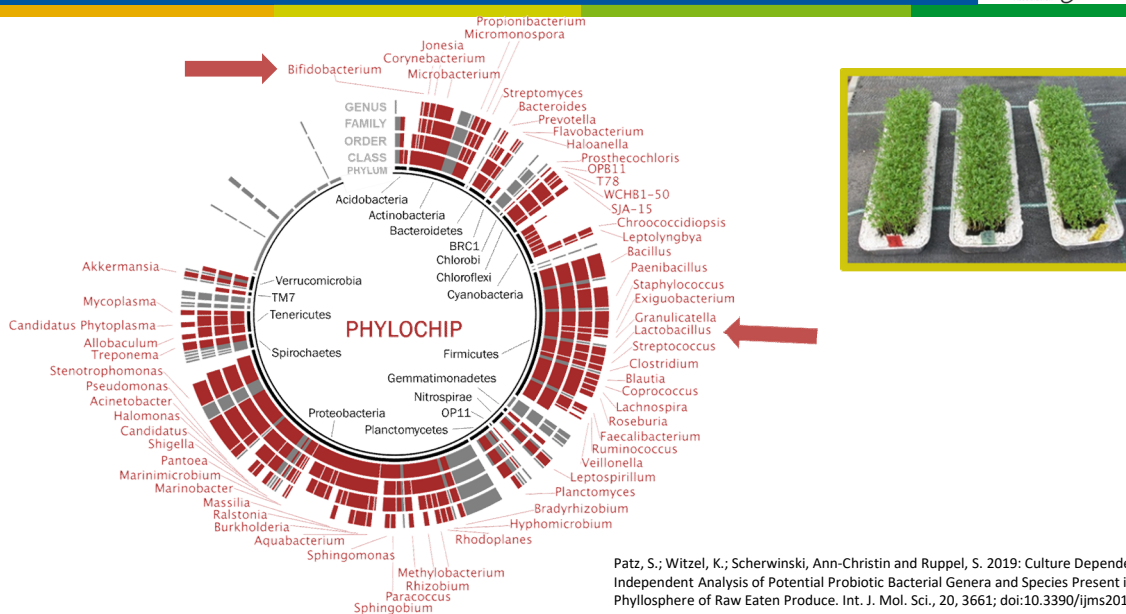
Endive



Lolo bionda

- Rhizosphere and soil microbial community significantly affects the plant available nutrient transfer (Nitrogen, Phosphate, and others) and the carbon cycle
  - Mineralization of organic matter
  - Biological nitrogen fixation from the air
  - Solubilization of rock phosphate

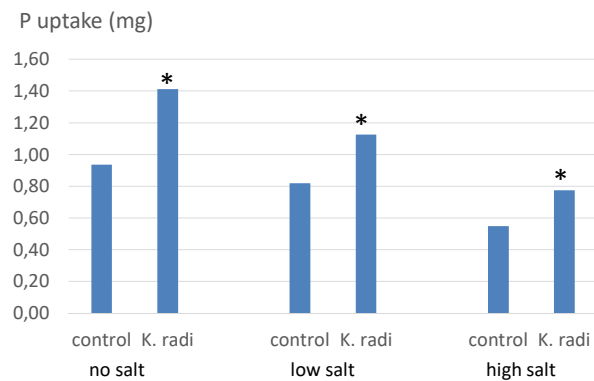
**Bacterial community composition of *Lepidium sativum***



### Tomato plant P-uptake after *Kosakonia radicincitans* inoculation



*Solanum lycopersicum* pot experiment fertilized with Camerunian Rock Phosphate and seed inoculation with  $10^8$  bacterial cells of *K. radicincitans*



### Assessing bacterial diversity



#### Culturable methods

- MPN analysis using serial dilution and cultivation on universal or selective media
- Community level physiological profiling

#### Molecular methods

- DGGE – Denaturing Gradient Gel Electrophoresis
- RFLP – Restriction Fragment Length Polymorphism
- ARISA – Automated Ribosomal Intergenic Spacer Analysis
- Microarray – PhyloChip
- Metagenome sequencing and comparison

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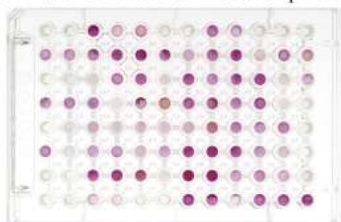
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## CLPP based bacterial community comparisons



### Substrate utilization diversity of the soil microbial biomass

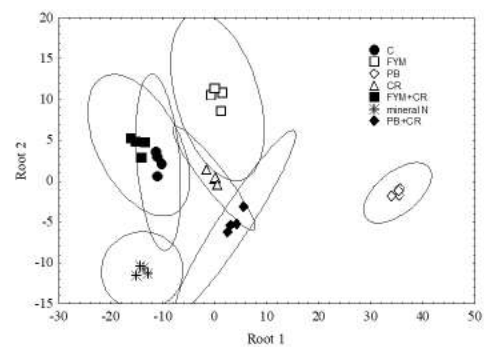
GN- und GP-BIOLOG microtiter plates



Diversity (H) calculated using Shannon Index, including substrate richness (S) and substrate evenness (E)

Spectroscopic analysis of substrate utilization patterns

Results of discriminant analysis of CLPP from prokaryotic communities after long-term differential fertilization



Ruppel, S., Torsvik, V. Daae, F.L., Øvreås, L and Rühlmann, J., 2007: Nitrogen availability decreases prokaryotic diversity in sandy soils. *Biol Fertil Soils* 43: 449-459.

## Assessing bacterial diversity



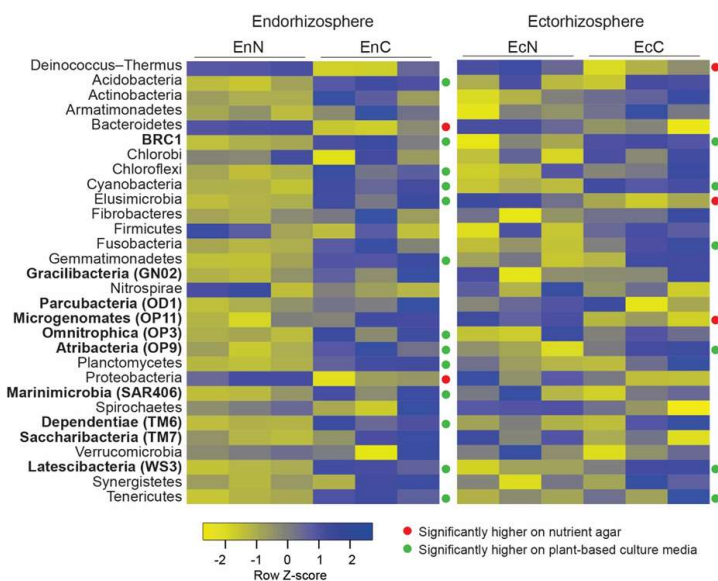
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## PhyloChip based bacterial community comparison



Heatmap representing the significant differences between the tested culture media (nutrient agar and plant-based culture medium) in respect of weighted abundance of the 30 phyla, representing more than 95% of all detected OTUs, in the maize compartments of endorhizosphere and ectorhizosphere.

Sarhan, M.; Patz, S.; Hamza, M.; Youssef, H.; Mourad, E.; Fayed, M.; Murphy, B.; Ruppel, S.; Hegazi, N. 2018: G3 PhyloChip analysis confirms the promise of plant-based culture media for unlocking the composition and diversity of the maize root microbiome and for recovering unculturable candidate divisions / phyla. *Microbes and Environments* 33, 317-325. ID ME18023.R2

## Metagenomics



- Metagenomic analysis to get a better understanding of the entire microbiome within an environmental sample, but be aware of:
  - Advantages & Disadv. Of 16S-Amplicon shotgun sequencing approach and whole MG-Sequencing (e.g. functional annotation, ...)
  - Setup of the experiment (hypothesis and biological replicates)
  - Sampling procedure (representative, mixture/ single parts, handling of the material, storage temperature, time span between sampling and further processing....)
  - Sample quality (Extraction method, efficiency in quantity and quality of DNA, RNA)
- Sequencing method affects the data quality and detected organism richness
- Annotation shall be done on experimentally proved datasets (quality of the databases)
- Algorithms and thresholds in statistical analysis affect the conclusions

