



Frontiers: Research Topic: Stenotrophomonas

Microbial networking for health issues

*Plant and Soil Microbiome
relevance for crop protection*

Gabriele Berg, AUSTRIA



Koninklijke Nederlandse Plantenziektkundige Vereniging

A) Introduction

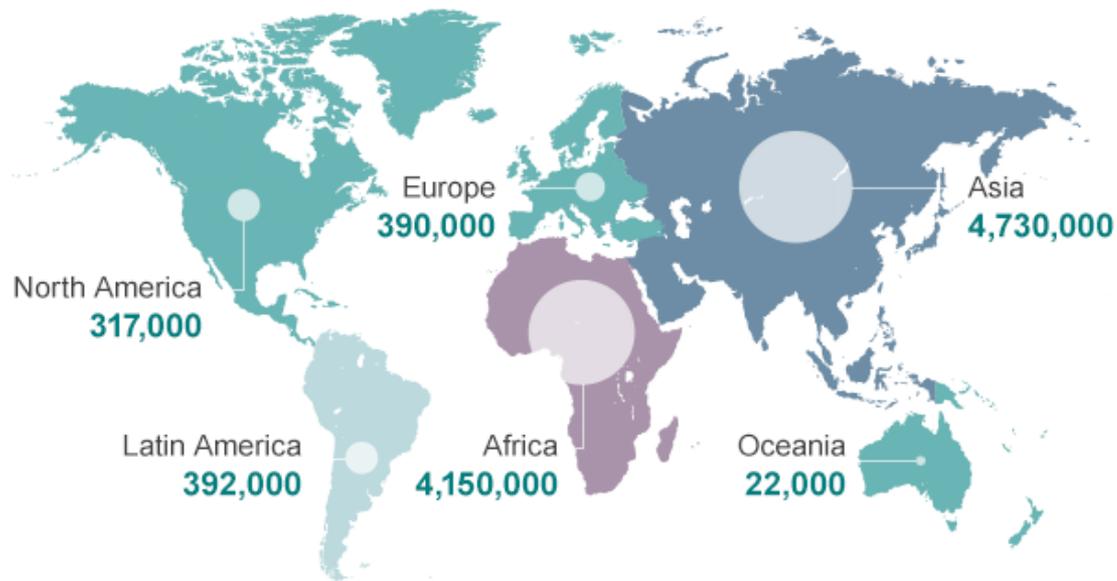
Increasing problems to control plant pathogens



- ✓ **Emerging pathogens**
- ✓ **Increasing resistance against pesticides**
- ✓ **Pesticide residues**
- ✓ **Non-target effects and environmental problems**

Increasing problems to control human pathogens

Deaths attributable to antimicrobial resistance every year by 2050



Source: Review on Antimicrobial Resistance 2014



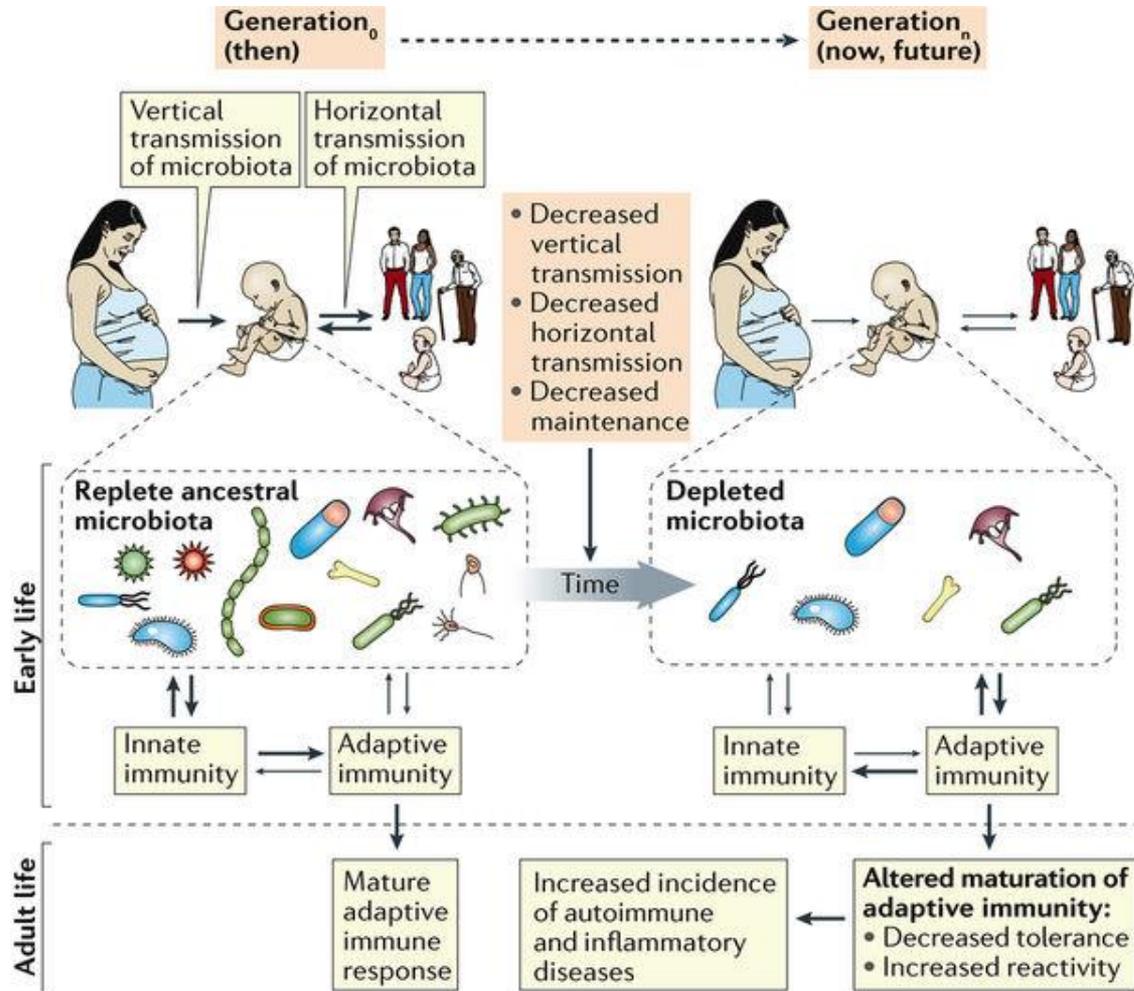
- ✓ **Increasing resistance against antibiotics**
- ✓ **Increasing number of chronic diseases**



**World Health
Organization**

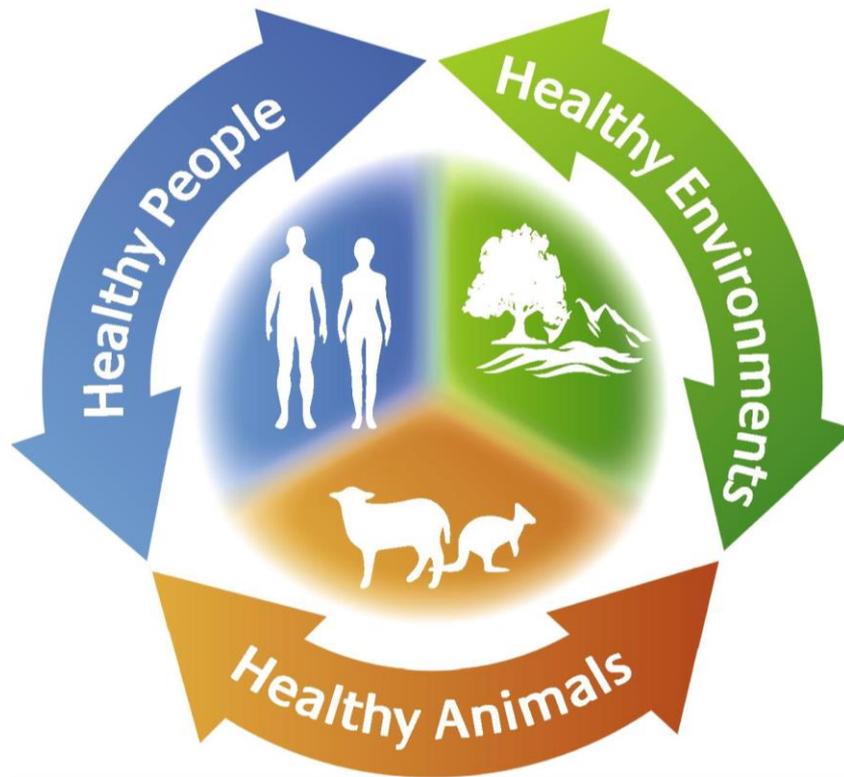
Missing microbes and human health

The theory of disappearing microbiota and the epidemics of chronic diseases by Martin Blaser (2017)



I. What role does diversity play in the agroecosystem?

II: What is the role of domestication for the plants?



III. What role does diversity play in human health?

Where we are? The Institute



Prof. Gabriele Berg

Leitung



Stellv. Leitung



Henry Müller

Sekretariat



Monica Genser

Universität Assistent/in



Christina Möller



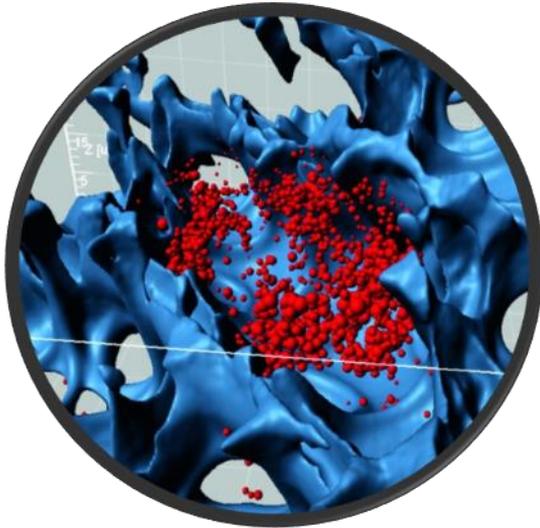
Armin Eracher



Environmental Biotechnology

Senior Scientist	 Maria Schmuck	 Maria Sensen	 Christin Zachow	Technische Assistentinnen	 Monika Schneider-Trampitsch	 Angelika Schaefer	 Barbara Fetz		
	Post Doc	 Stefan Liebming	 Daria Rybakova		 Martina Köberl	 Tomislav Cernava	 Ines Aschenbrenner	Doktorand/in	 Alexander Mahner
		 Julian Taffner	 Melanie Obermeier		 Rocel Amor Ortega	 Eveline Adam	 Alessandro Bergna		 Barbara Gstöttenmayr
Projektmitarbeiter		 Mohammad Etemadi	 Alexander Fuchs	Master Student/in	 Sabine Erschen	 Christina Laireiter	 Kathrin Hölzl		 Stephanie Holck
	 Christoph Provasnek	 Maria Bernhart	 Birgit Wassermann		 Pascal Müller	 Lisa Krug	 Peter Kusstatscher	 Verena Kühma	
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Our Mission: Microbiome research and Microbiome-based solutions for health



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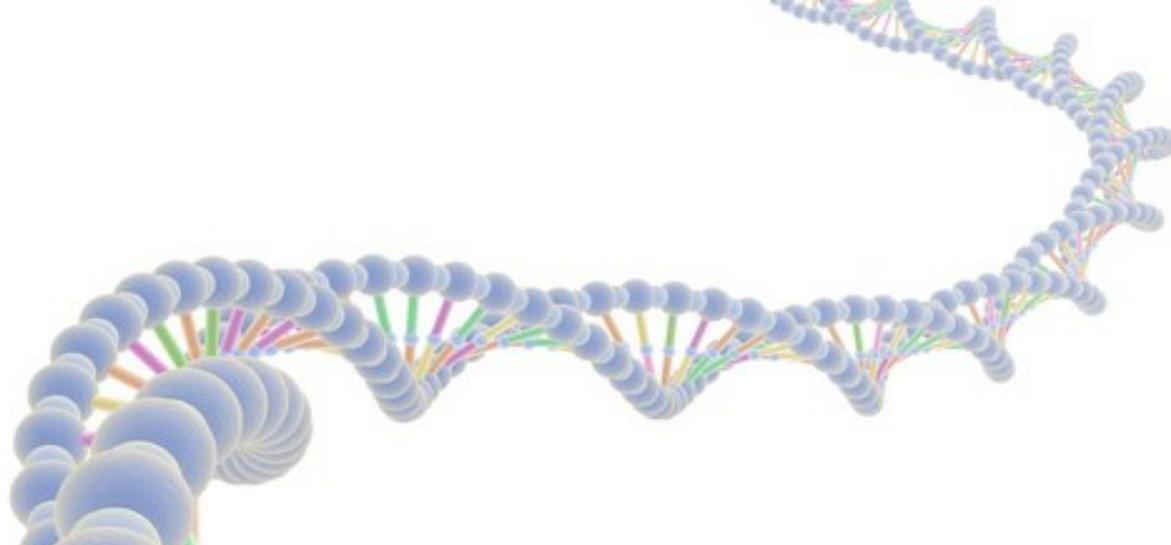
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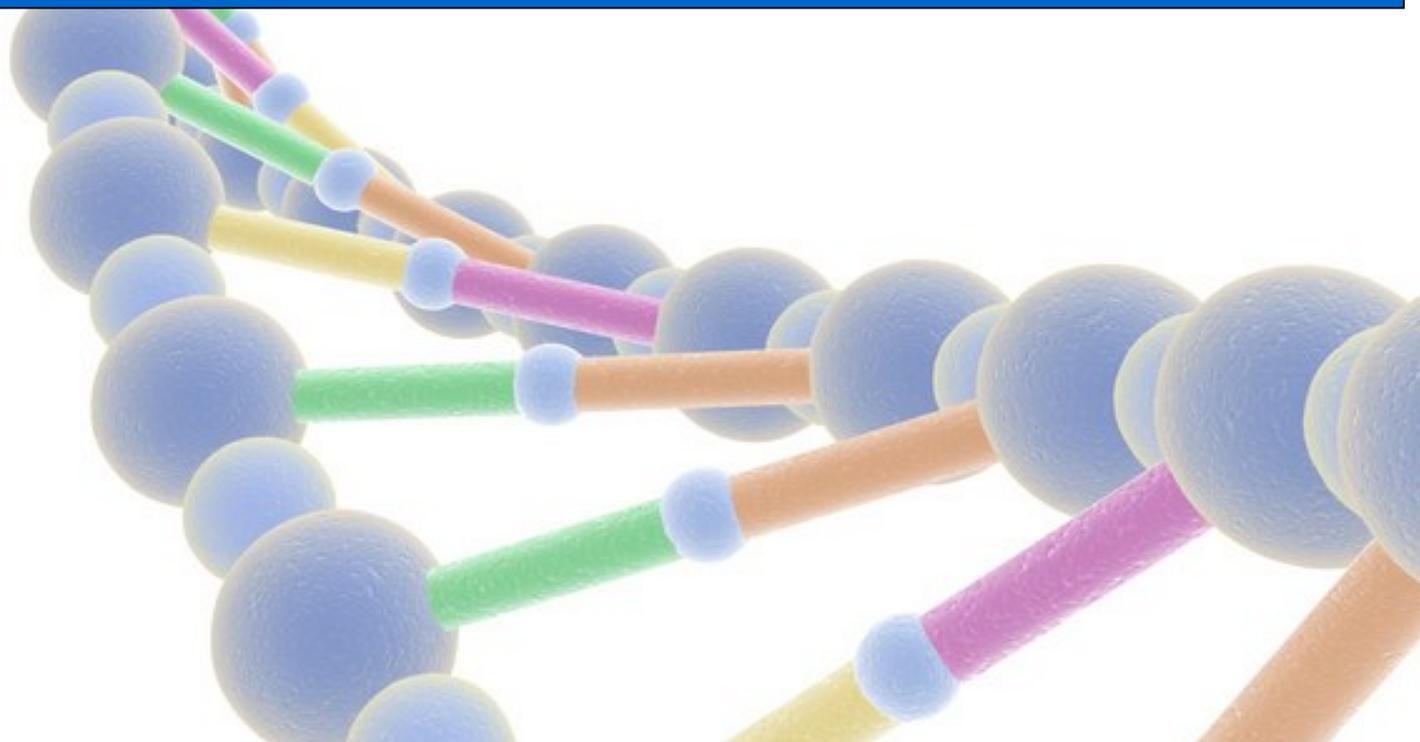
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B) Results





I. What role does diversity play in the agroecosystem?

Biocontrol of soil-borne pathogens

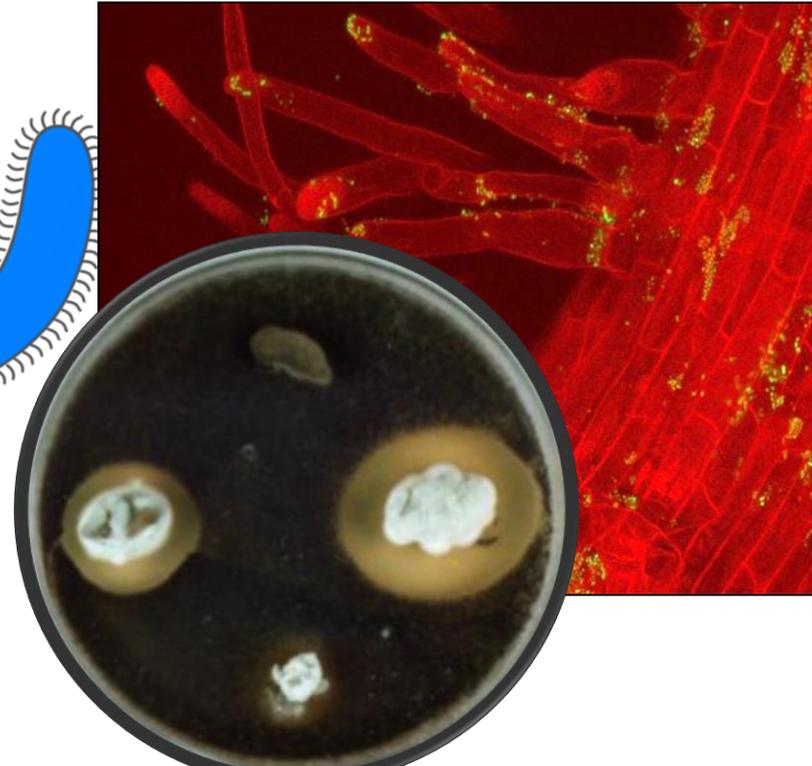
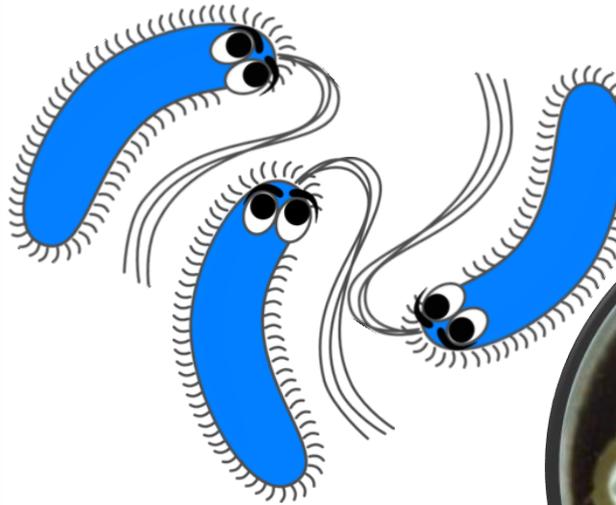
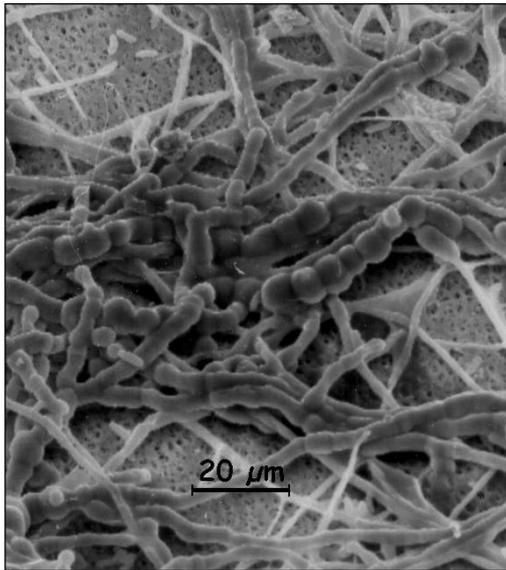


BIOCOMES

New biological control products
for sustainable farming and forestry

The logo for BIOCOMES consists of three main elements: a blue cartoon microorganism with large eyes and a long tail, a green stylized letter 'B' containing four white arrows pointing upwards and to the right, and the flag of the European Union (a blue rectangle with twelve yellow stars arranged in a circle). The text 'BIOCOMES' is written in a bold, black, sans-serif font, and the tagline 'New biological control products for sustainable farming and forestry' is written in a smaller, black, sans-serif font below it.

Verticillium wilt – the disease



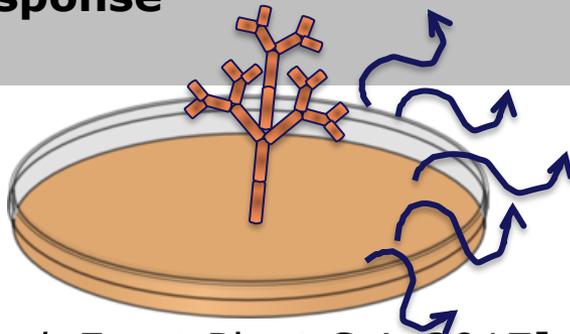
➤ Biocontrol solution needed?

Oilseed rape: biocontrol of *Verticillium* wilt

Paenibacillus polymyxa Sb3-1 and *Verticillium longisporum* ELV 43 have an ongoing dialogue through their VOCs:

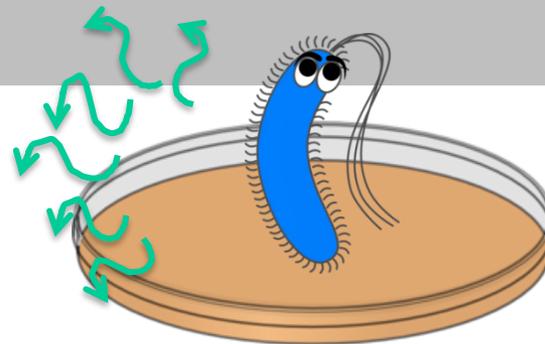
Verticillium

- **Growth reduction**
- **Constant** production of the **antimicrobial** (e.g. 1-Butanol) & **PGP** (e.g. Acetoin) VOCs
- **Upregulation** of some **antimicrobial volatile** substances
- **Downregulation** of the **protein and DNA components** and **cellular stress response**



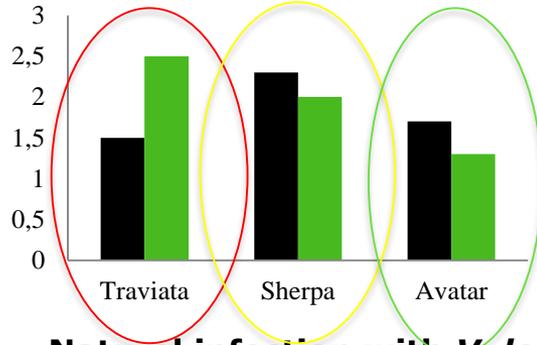
Paenibacillus

- **Constant growth**
- **Constant** production of the **antimicrobial** (e.g. Pyrazines) & **PGP** (e.g. 2,3-Butandione) VOCs
- **Downregulation** of some **antimicrobial volatile** substances
- **Upregulation** of the **protein and DNA** components

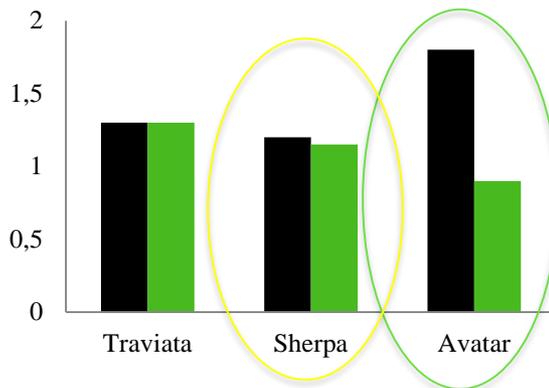


The biocontrol effect was cultivar-specific

Artificial infection with *V. longisporum*



Natural infection with *V. longisporum*



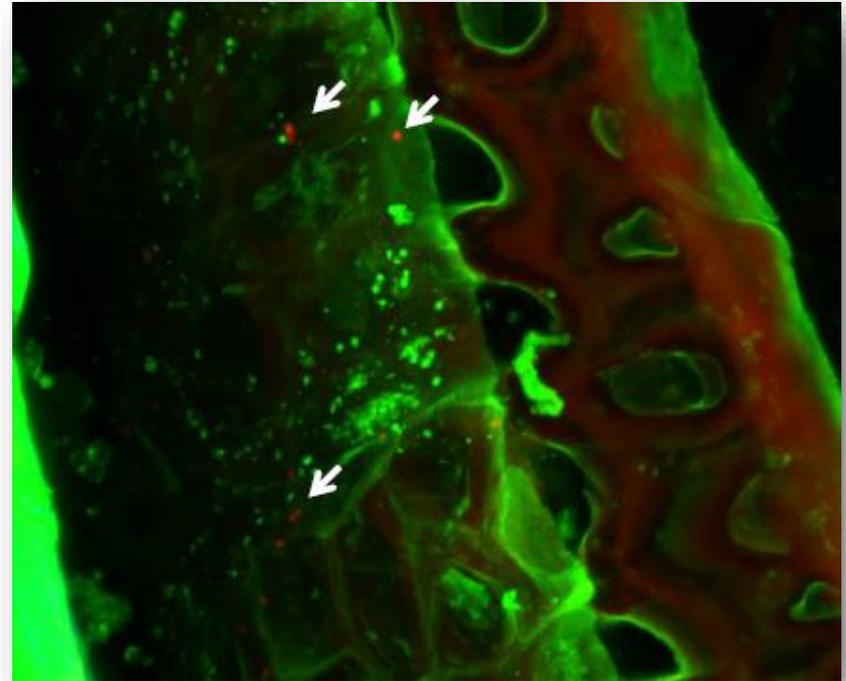
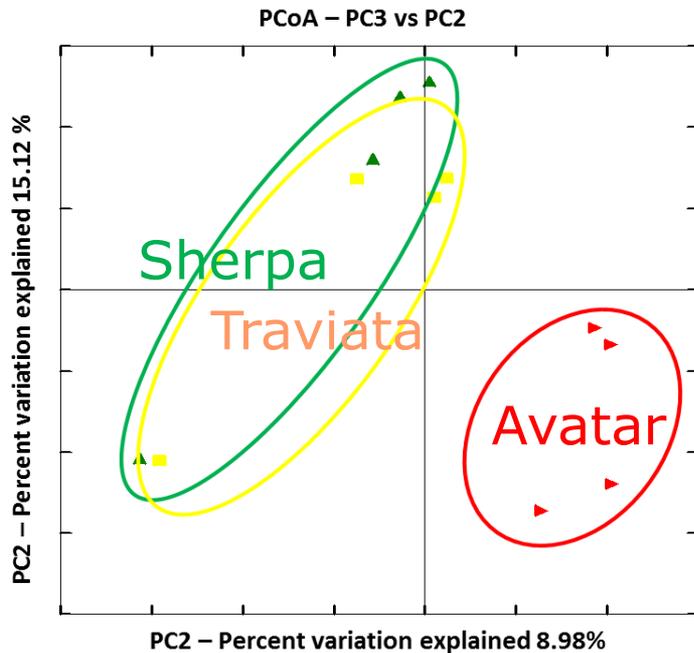
■ Untreated control

■ + *P. polymyxa* Sb3-1

- Increase of the disease rate in Traviata
- No differences due to Sb3-1 in Sherpa
- Disease control by Sb3-1 in Avatar



The seed microbiome was cultivar-specific

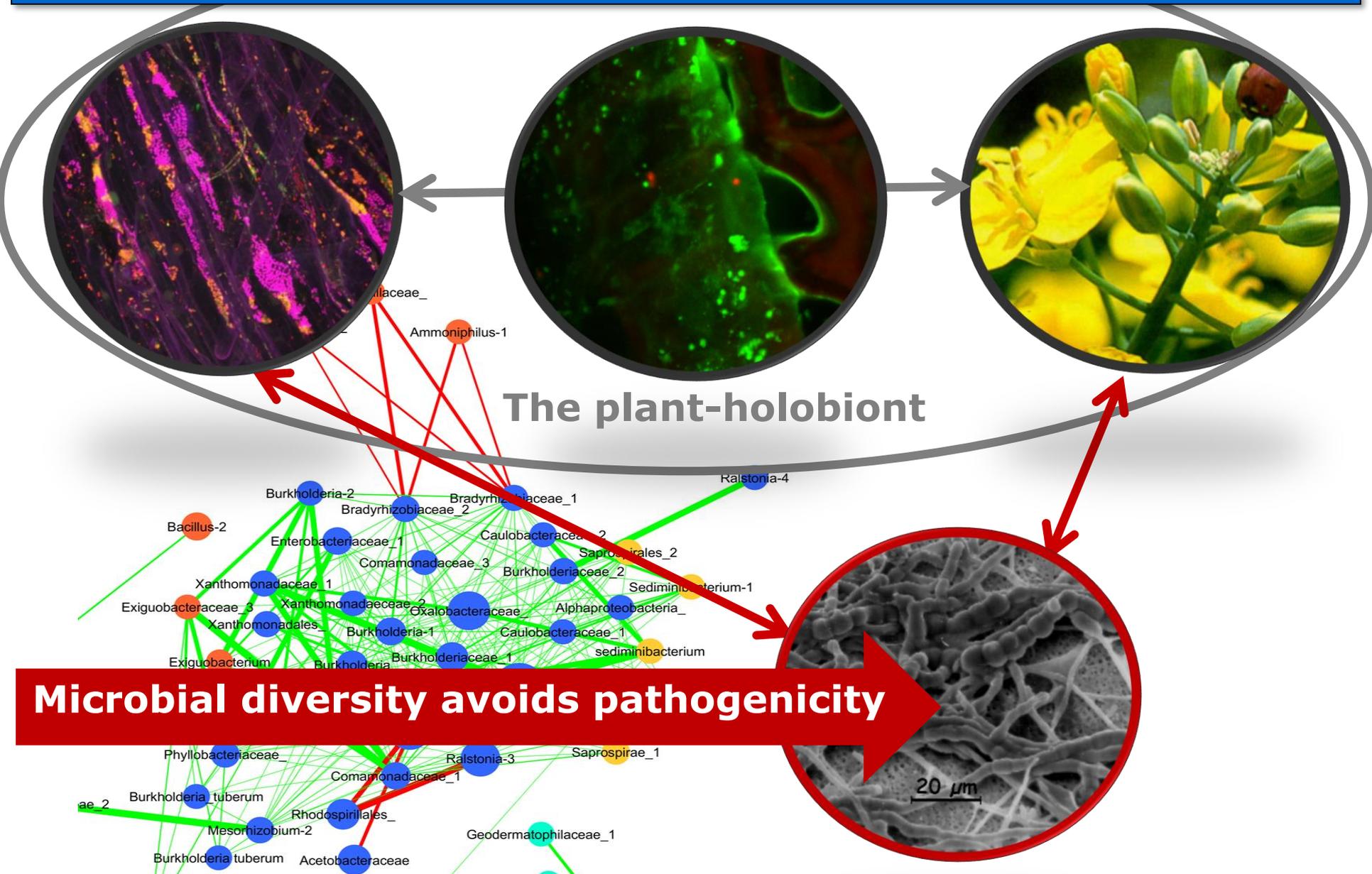


Avatar seed microbiome:

- lower alpha diversity
- increased amount of some potential pathogens (e.g. *Ralstonia*: 13.1%!)
- decreased amount of beneficial phyla

[Rybakova *et al.* Microbiome 2017]

CONCLUSION I

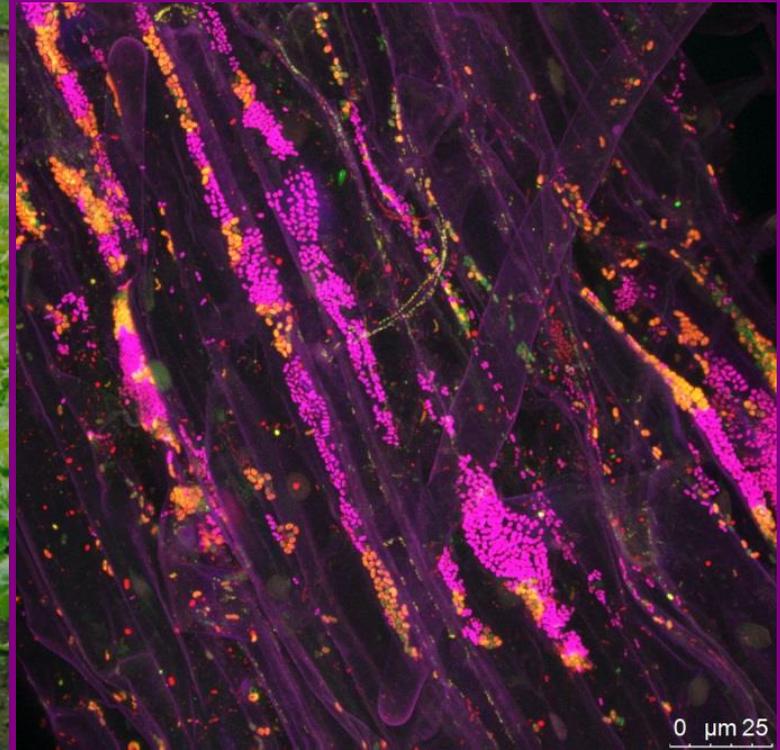


[Berg *et al.* Microbiol Biotech 2015, J Exp Botany 2016, FEMS Microb Ecol 2017]

II: What is the role of domestication for the plant microbiome?



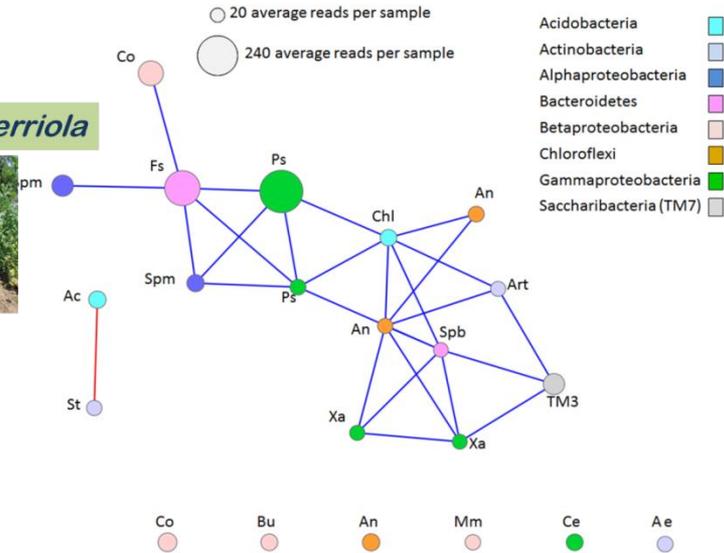
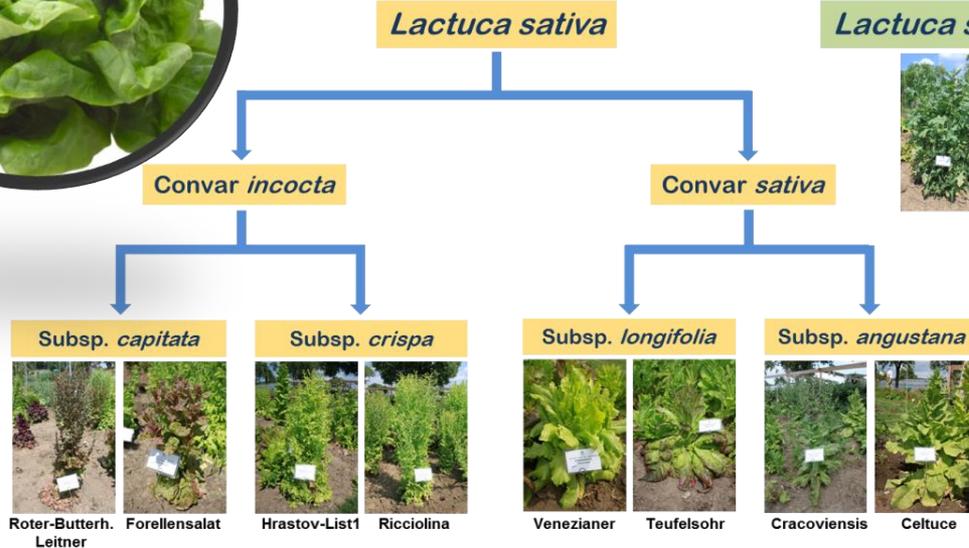
Lettuce: Microbial networking for health



The Lettuce project (EU)
Armin Erlacher, Massimiliano Cardinale
Martin Grube (KFU Graz)



Lettuce: The impact of domestication

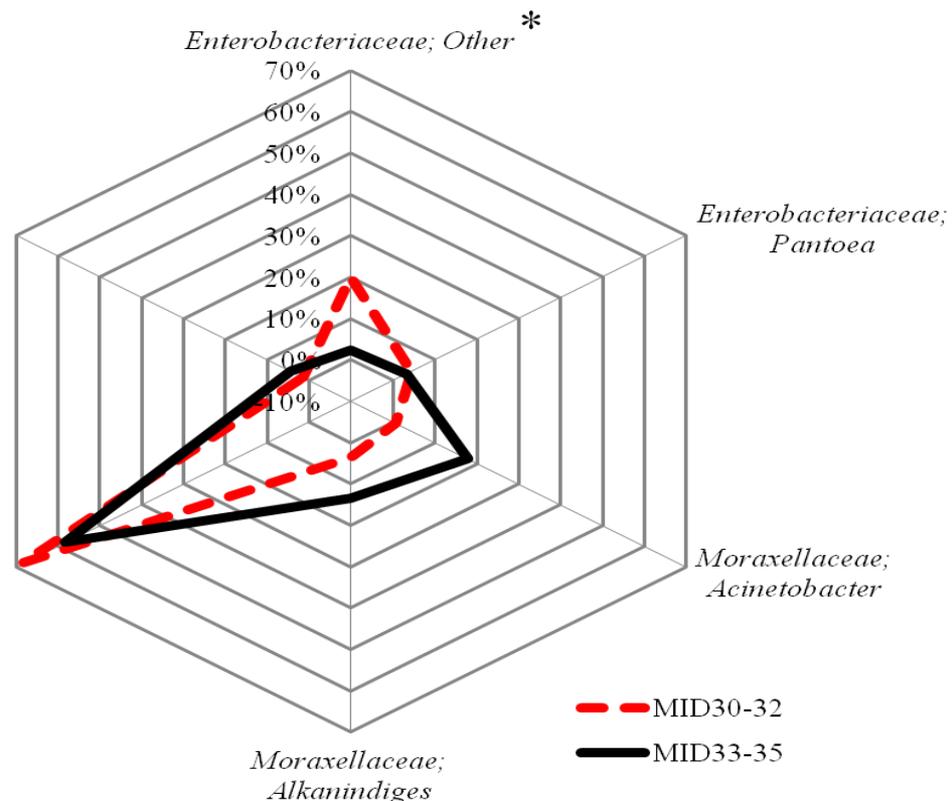
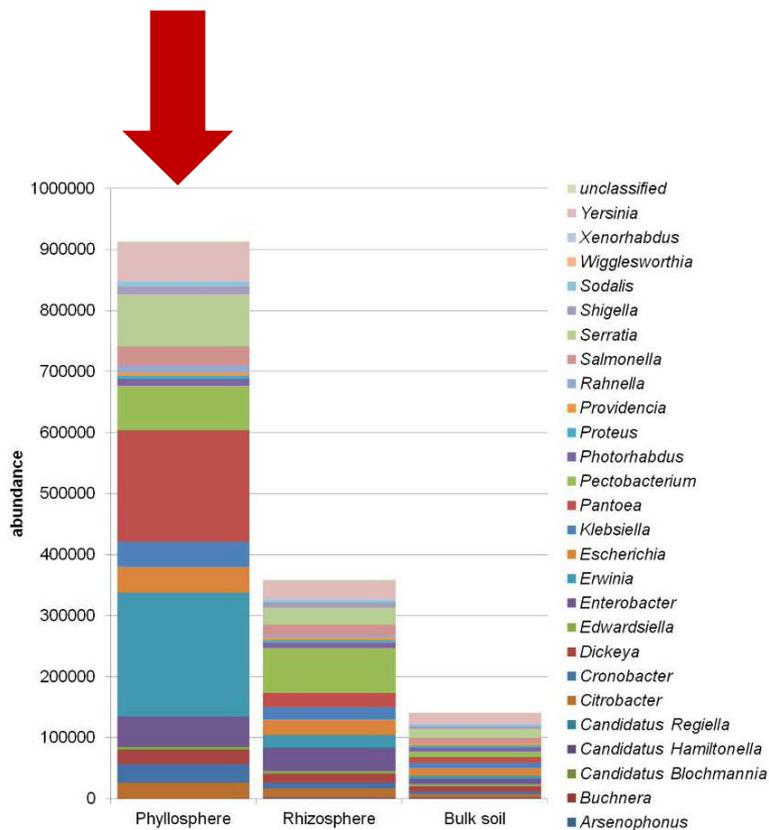


- significant differences at species and cultivar level
- 12.5% cultivar-specific bacteria; 49% core microbiome

Domestication (breeding) lead to bacterial diversification in lettuce root system.

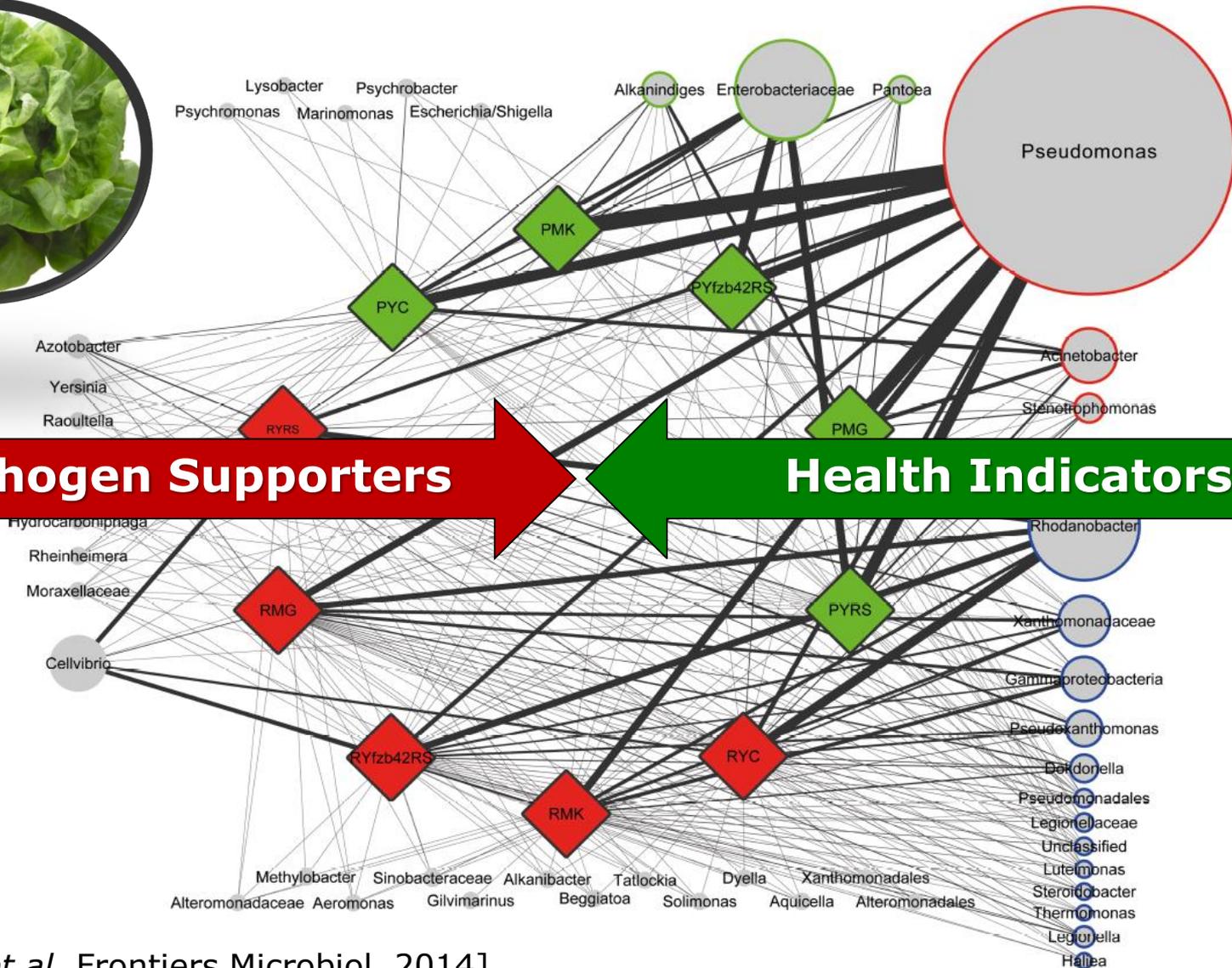
Loose network structure was observed – health relevant

Lettuce: The lettuce microbiome and disturbance



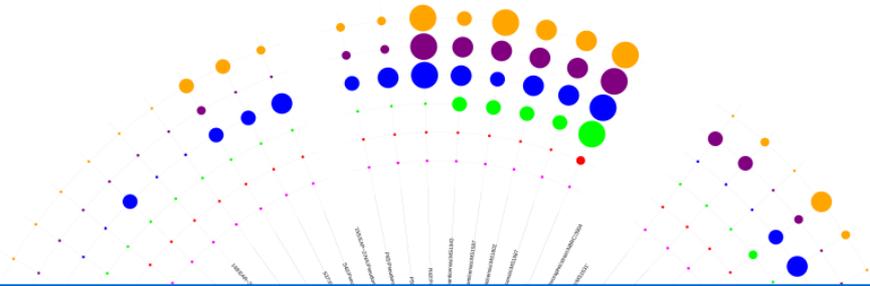
Disturbances increase the abundance of *Enterobacteriaceae*: *Enterobacter* in lettuce and *Escherichia* and *Pantoea* in rucola.

Lettuce: Looking for biocontrol agents

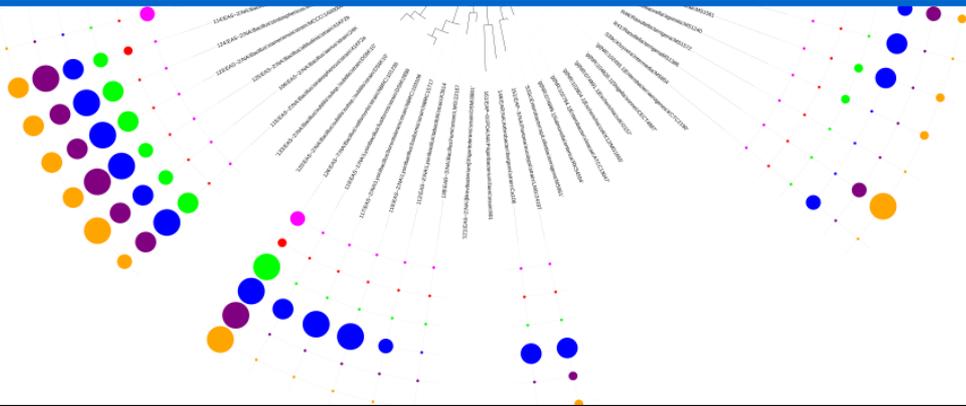


[Erlacher *et al.* Frontiers Microbiol 2014]

Lettuce: Biocontrol solutions



The antagonistic spectrum comprise mainly plant-beneficial *Enterobacteriaceae* as promising biocontrol agents.



Novel broad-spectrum antagonists

Untreated (negative control)

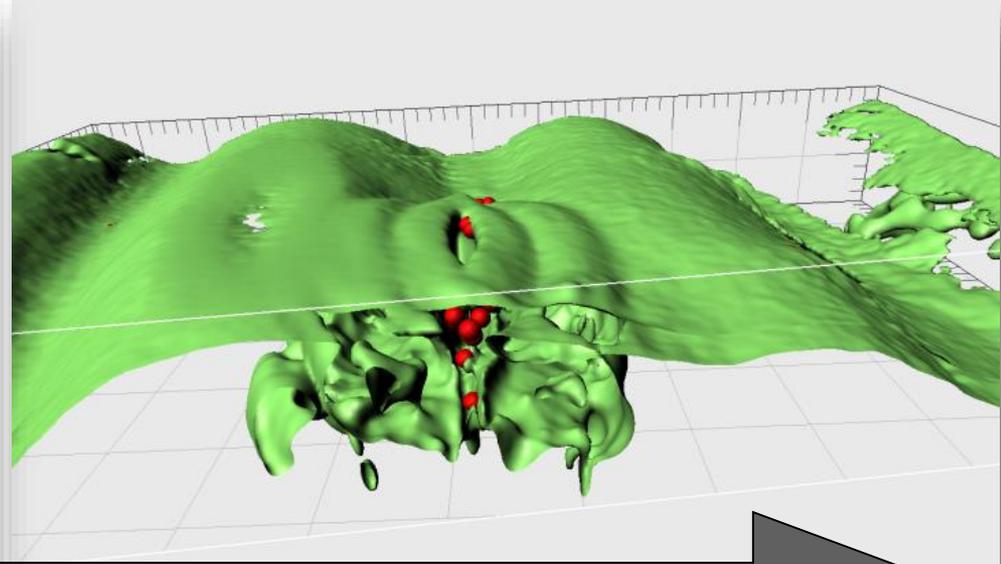


Co1-6 inoculated

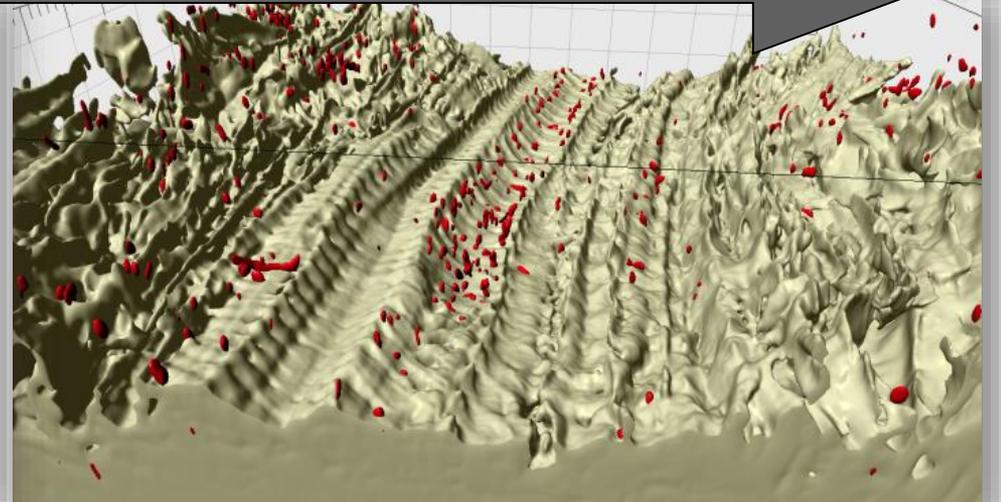


[with Leo van Overbeek]

Lettuce: vizualizing the lettuce microbiome



Natural vaccination by endophytes?



Pumpkin: Microbial networking for health



The pumpkin microbiome project (FFG)
Eveline Adam, Henry Müller, Maria Bernhardt
Maria Winkler (Saatzucht Gleisdorf)

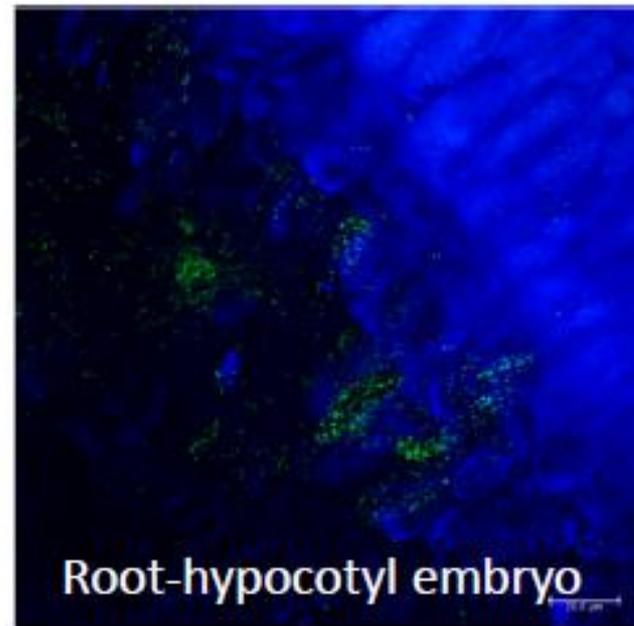
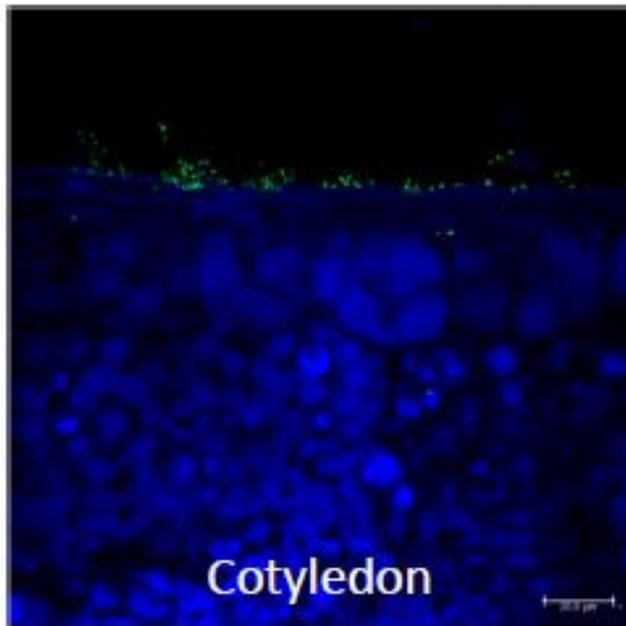
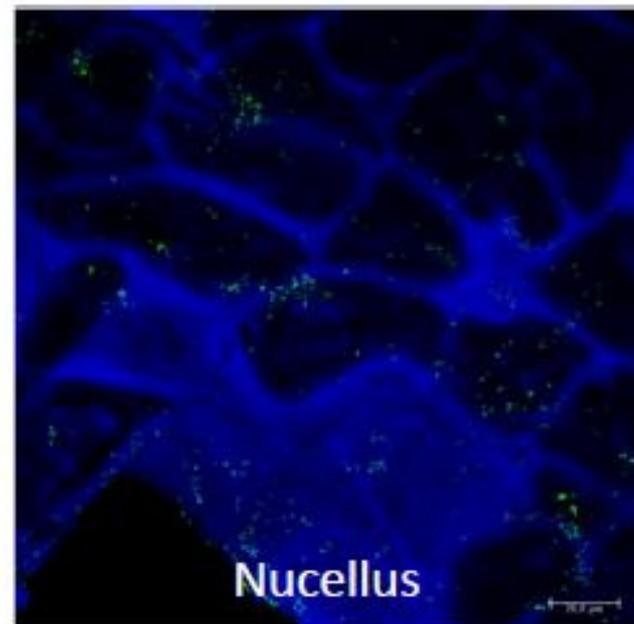
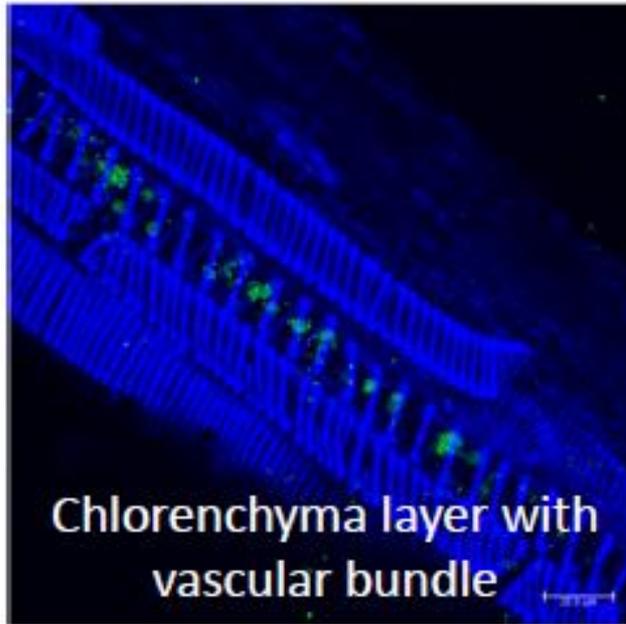


The pumpkin microbiome

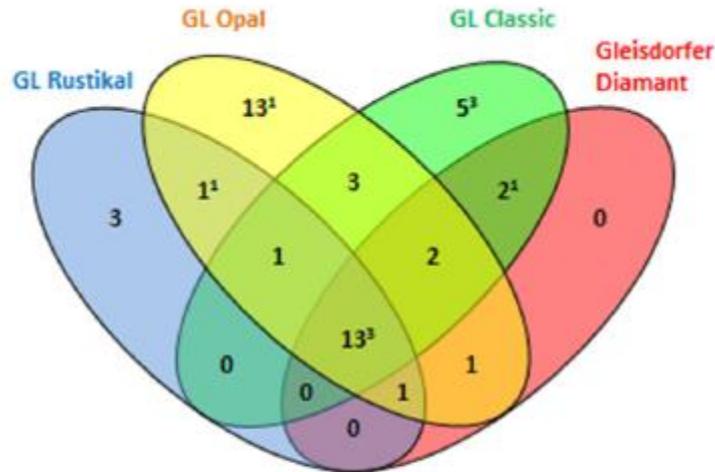
Type	Denomination	Pedigree	Geographic origin	Seed samples
Homozygous inbred lines	Line A - D	-	Austria	
Single cross hybrid	Gleisdorfer Diamant	Line A x Line B	Austria	
Three-way cross hybrids	GL Opal GL Rustikal	Gl. Diamant x Line C Gl. Diamant x Line D	Austria	
Population cultivar	GL Classic	-	Austria	
Single cross zucchini hybrid	Naxos	-	Netherlands	
Segregating breeding lines	Line E - I	-	Germany, Slovenia, China	

[Adam *et al.* Plant and Soil 2016]

The pumpkin microbiome



The pumpkin microbiome



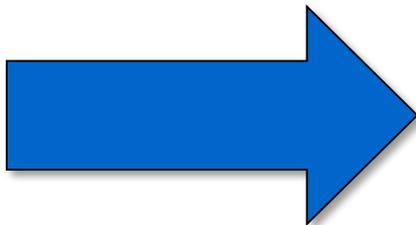
Unique and shared *Enterobacteriaceae* OTUs of the four cultivars 'GL Rustikal', 'GL Opal', 'GL Classic' and 'Gl. Diamant'. Counts of *Erwinia* sp. are in superscript.



Huss et al., 2009

P. carotovorum (*Erwinia carotovora*) causes soft rot of fruits.

Connection of higher diversity with the displacement and competition of *Enterobacteriaceae*?



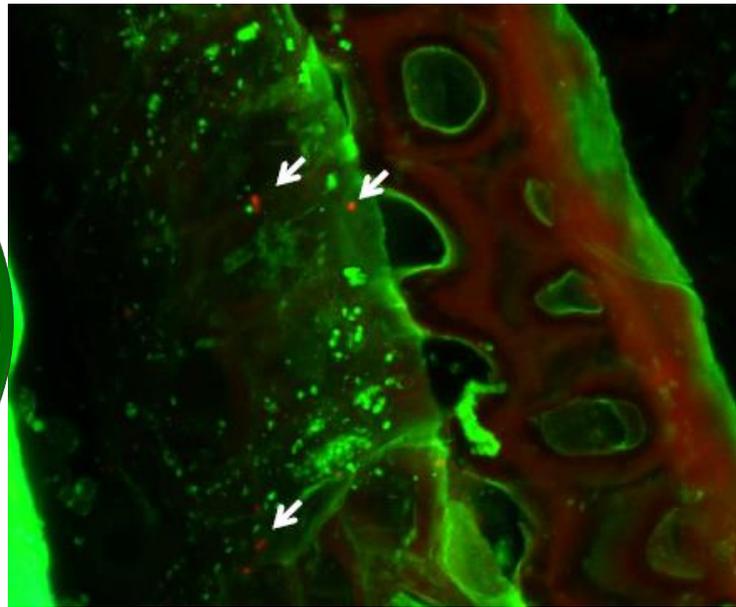
Germination rate field trials 2016

Control untreated:	39%
Maxim XL/Captan stripper:	89%
Microbial consortium without chemical stripper:	90%



Pumpkin plants 16 days after sowing in the field. A, B: untreated negative control showing low plant vigor; C: plant after 'Peposan' seed treatment.

CONCLUSION II



- **Seeds transferred a core microbial diversity**
- **Domestication changed the seed and plant microbiome**

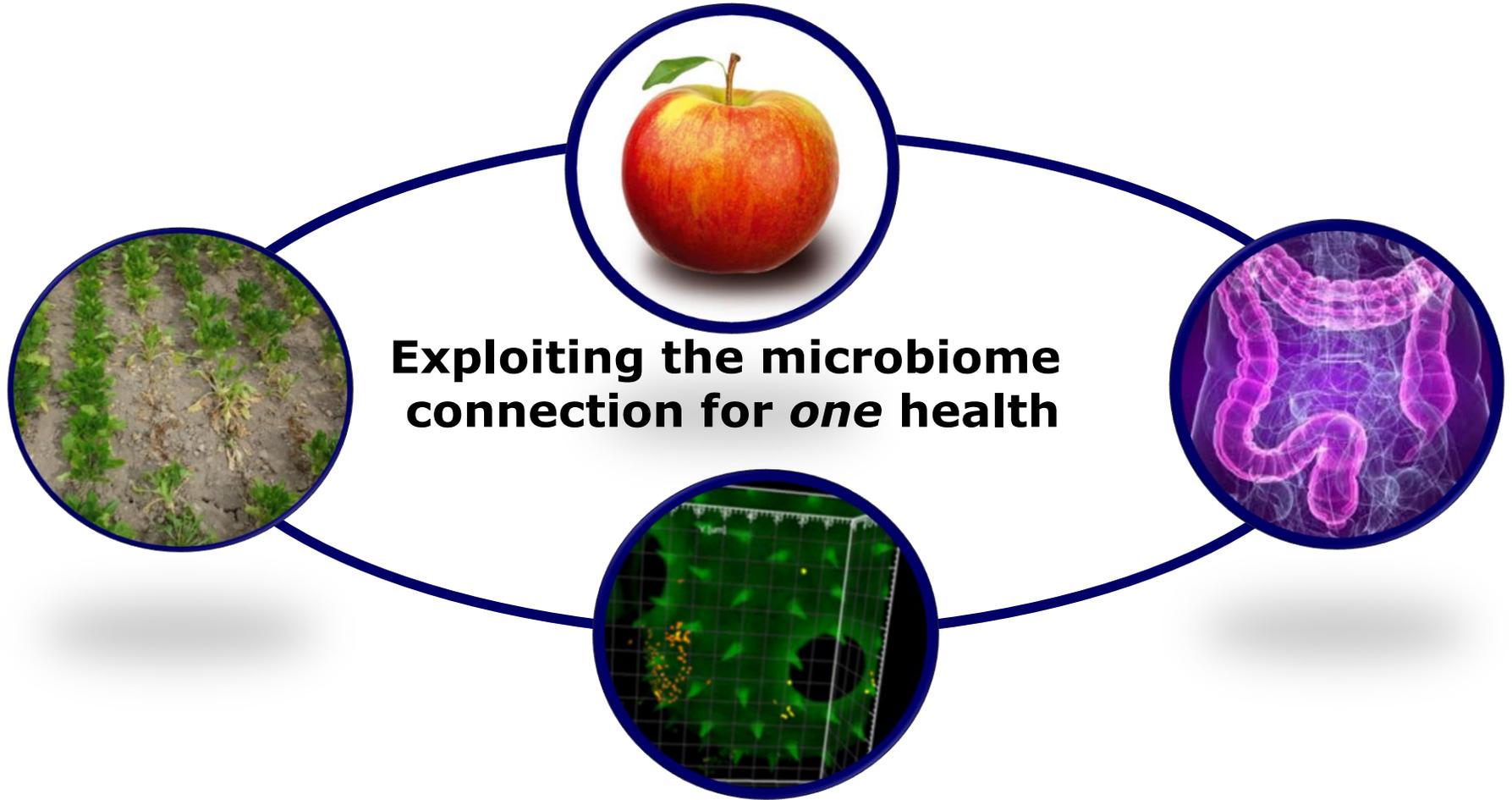
Shown for other crops, e.g.

- **maize (Peiffer *et al.*, 2013)**
- **barley (Bulgarelli *et al.*, 2015)**
- **bean (Pérez-Jaramillo *et al.*, 2016, 2017)**



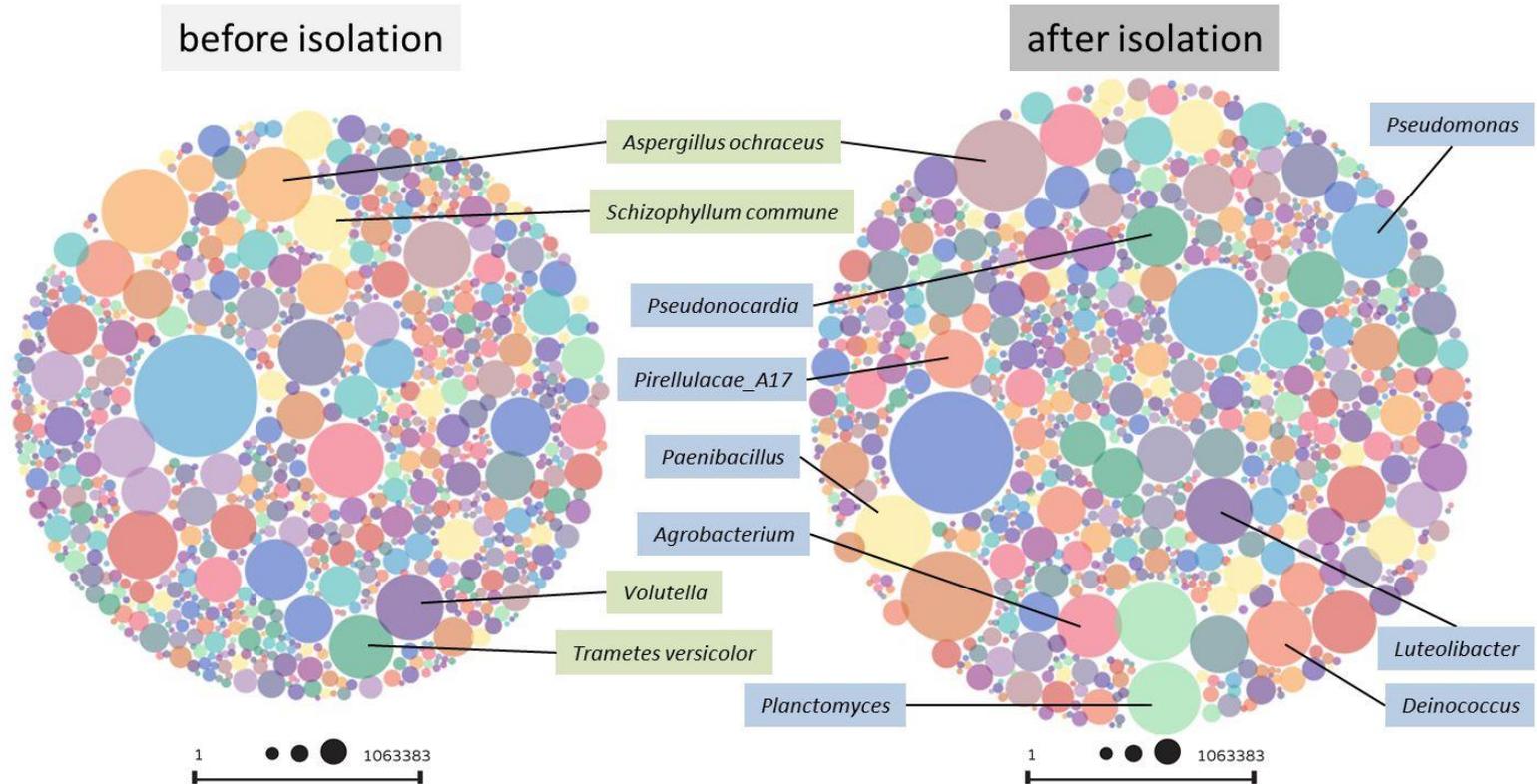
III. What role does diversity play in human health?

The microbiome connection



[**Microbiome connections:** Davis *et al.* Nature 2014; Qian *et al.* Indoor Air 2012; Mahnert *et al.* Frontiers Microbiol 2015]

The plant microbiome as source for beneficials

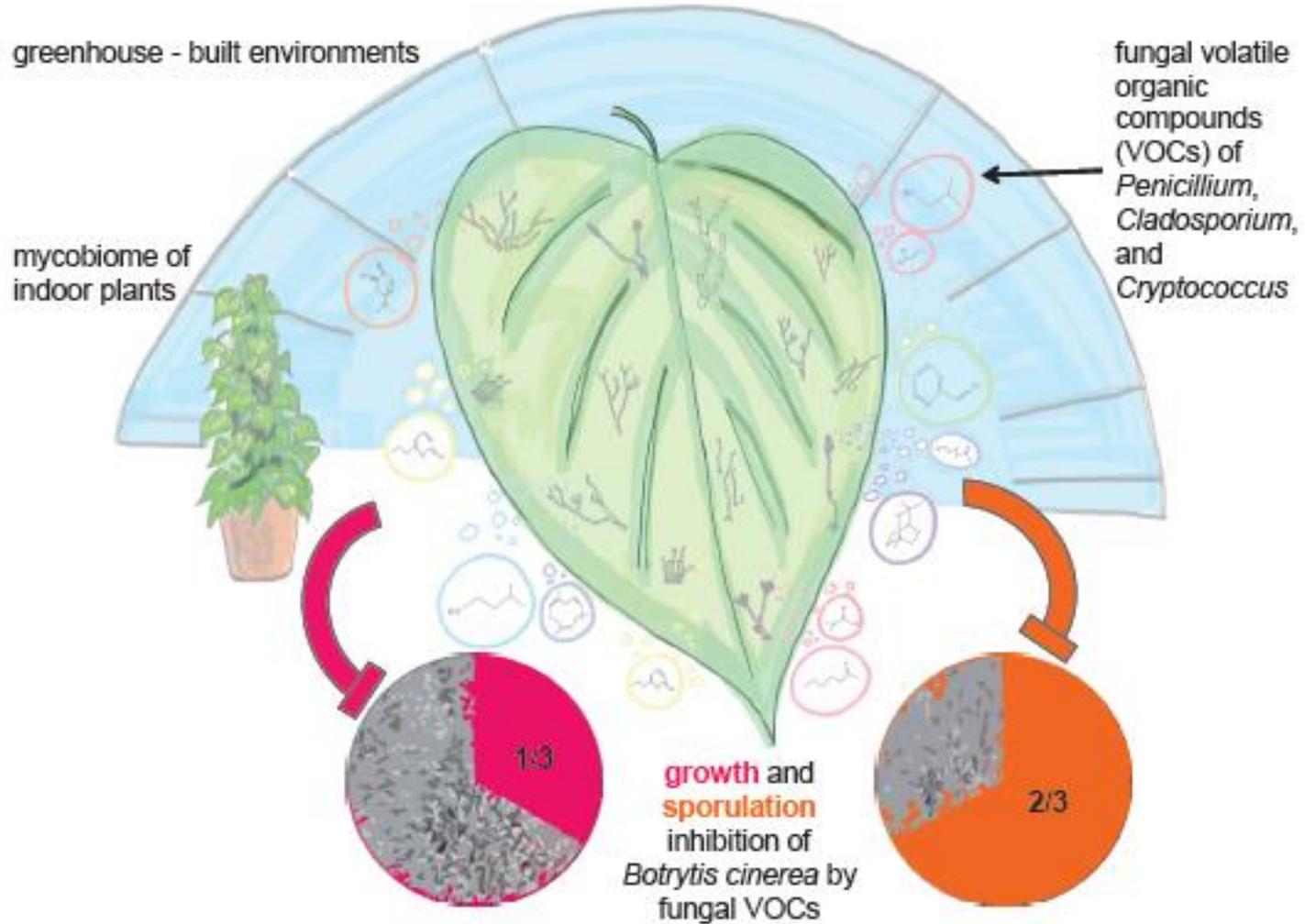


[Mahnert *et al.* Frontiers 2015]

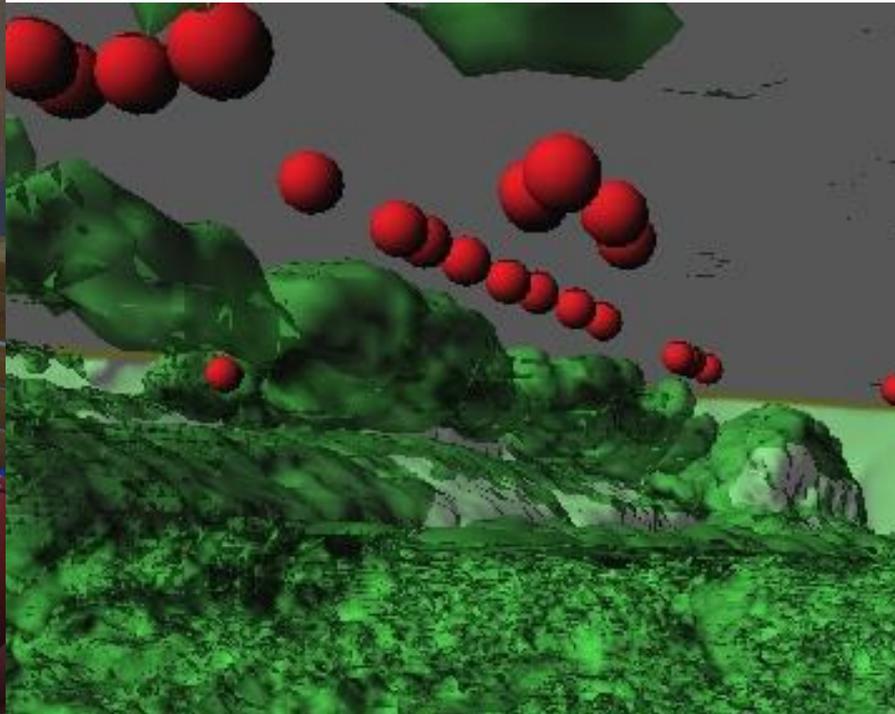


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The plant microbiome as source for beneficials



B) Plants – gut microbiome diversity



The banana microbiome

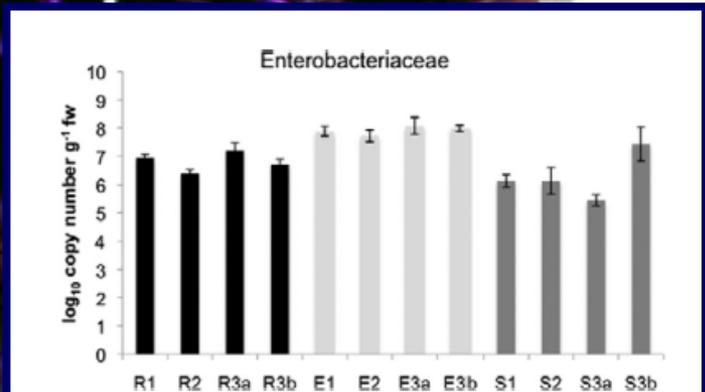
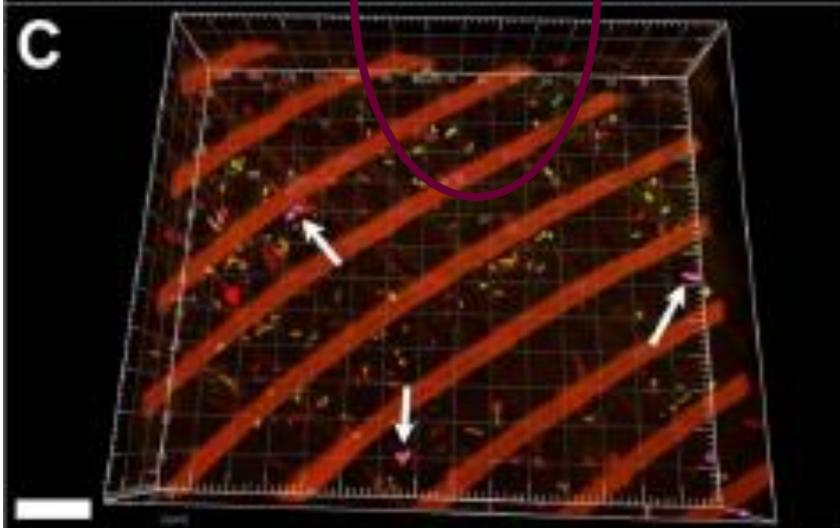
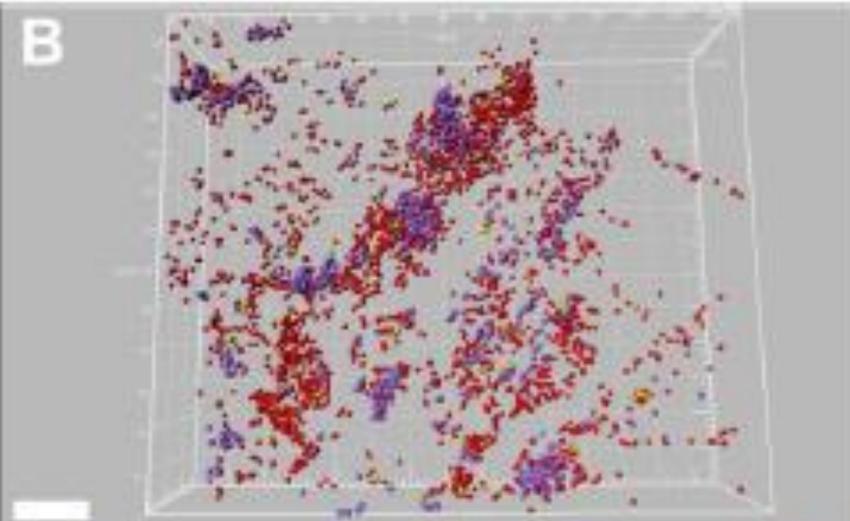
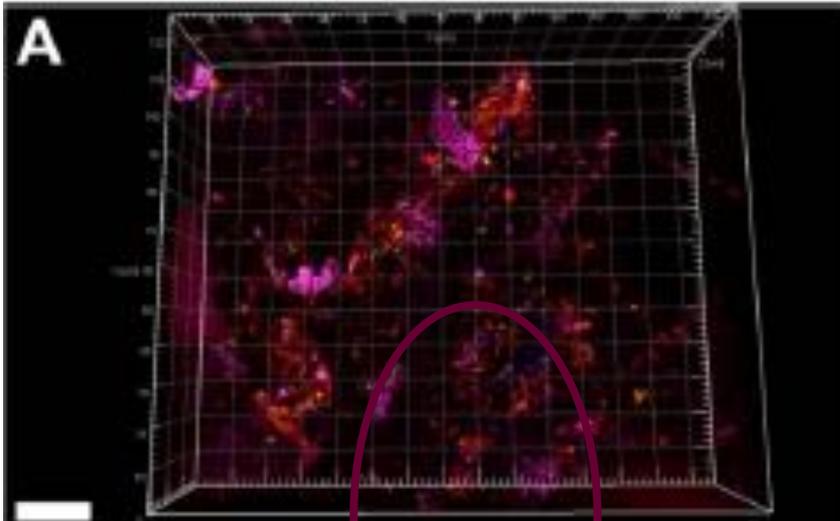
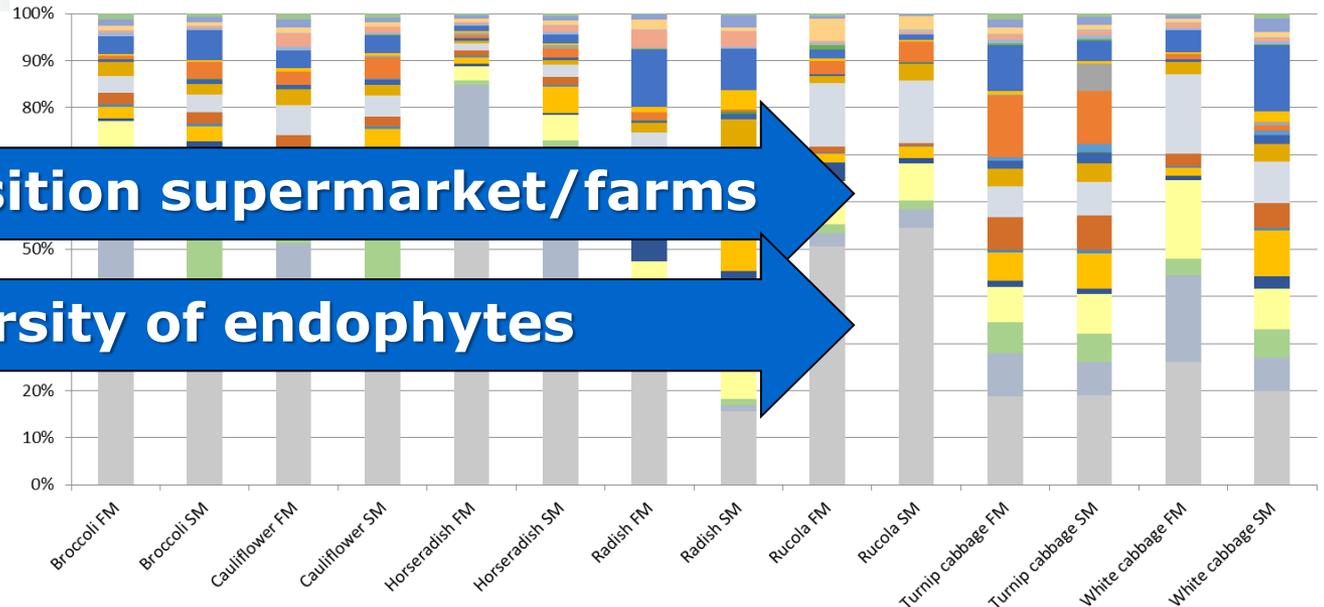


FIG 1 16S rRNA copy numbers per gram of fresh weight (g fw^{-1}) determined by *Enterobacteriaceae*-specific quantitative PCR. Log₁₀ values are shown for the rhizosphere (R), the endosphere (E), and soil (S) for the four investigated field sites: field 1, no herbicide treatment, no agro-forest; field 2, intensive herbicide treatment, no agro-forest; field 3a, no herbicide treatment, agro-forest; and field 3b, no herbicide treatment, no agro-forest. Error bars indicate confidence intervals at $P = 0.005$.

Gut-beneficial endophytes from *Brassicaceae*



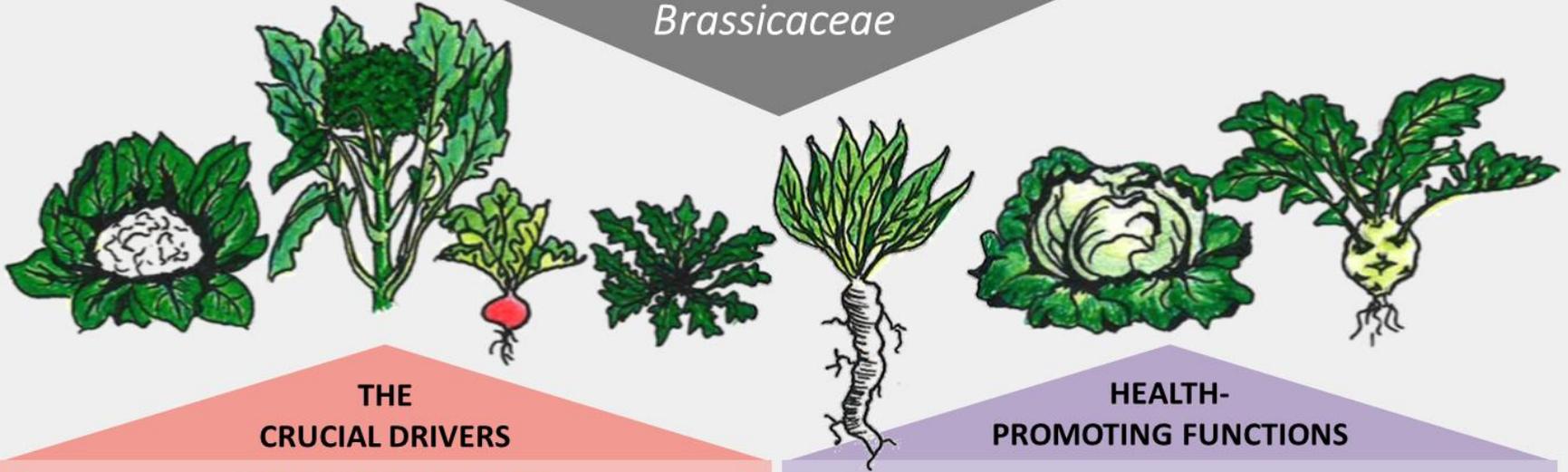
Distinct composition supermarket/farms

High diversity of endophytes

- Pseudomonadales
- Enterobacteriales
- Sphingomonadales
- Burkholderiales
- Xanthomonadales
- Actinomycetales
- Fimbrimonadales
- Cytophagales
- Flavobacteriales
- Spingobacteriales
- Saprospirales
- Herpetosiphonales
- Cyanobacteria
- Bacillales
- Lactobacillales
- Caulobacteriales
- Rhizobiales
- Rhodobacteriales
- Rhodospirillales
- Methylophilales
- Aeromonadales
- Alteromonadales
- Verrucomicrobiales
- Deinococcales

Gut-beneficial endophytes from *Brassicaceae*

The microbiome of raw-eaten
Brassicaceae



THE CRUCIAL DRIVERS

1. THE PLANT GENOTYPE

determines the microbiome of all plant tissues

2. THE PROCESSING PATHWAY

has only a vegetable type-specific effect on the microbiome

3. THE RARE GROUPS

are decisive for the microbial differences

HEALTH- PROMOTING FUNCTIONS

1. MYROSINASE-ACTIVE ENDOPHYTES

in raw eaten plant parts

2. PUTATIVE BIOLOGICAL CONTROL AGENTS

especially in naturally processed vegetables

LINKING FUNCTIONS WITH METAGENOMIC DATA

Myrosinase-active bacteria are enriched in GLS-secreting tissues of *Eruca sativa* and *Brassica napus*

Conclusion



- **The plant-associated microbial diversity is a key for plant and human health issues**
- **Supplementary biologicals can enhance microbial diversity and avoid pathogen outbreaks**
- **We have to re-think sterility and microbial diversity**