A healthy plant microbiome for healthy food and feed (production)

70th ed. EAAP, Ghent

28 August 2019, Leo van Overbeek

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100years
1918 — 2018

HUPLANT CONTROL

Cross kingdom pathogens

Fusarium spp.

Anna van Diepeningen, WUR, NL

Topics

- Cross Kingdom species involved in mycotoxin/ alkaloid production
- Endophytes - microbes living inside plants; introduction to the Plant Micrbiome
- The Plant Microbiome
- Circular agriculture
- Antibiotic resistance and genetic mobility of (acquired) resistance genes

Fungi and Bacteria in plants and animals

Potential human/ animal pathogens in plants

- *Fusarium*
- *Penicillium*
- *Aspergillus*
- *Trichoderma*

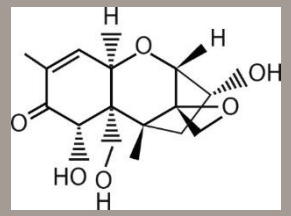
Ascomycota

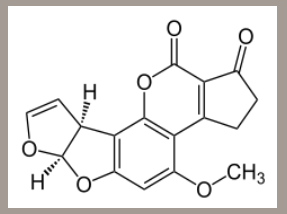
- *Enterobacteriaceae*
- *Stenotrophomonas*
- *Firmicutes (Bacillus)*
- *Bacteroidetes*

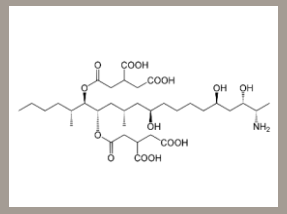
Mycotoxins in cattle feed (silage) and crop residues

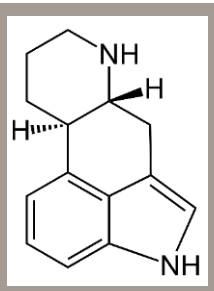
Trans-Kingdom Fungi

- Deoxynivanol (DON)
- Aflatoxin
- Fumonisin and Ochratoxin
- Ergot alkaloids









Mycotoxin producers

Mycotoxin	Fungal species	Crop/ product
Deoxynivalenol	*Fusarium graminearum*, *F. culmorum*	Corn, wheat, oats barley
Aflatoxin	*Aspergillus flavus*, *A. parasiticus*	Peanuts, corn
Fumonisin	*Fusarium verticilloides*, *F. proliferatum*	Corn, rice, sorghum
Ochratoxin	*Aspergillus ochraceus*, *A. niger, A. carbonarius*, *Penicillium verrucosum*	Cereals, coffee, dried fruits

Ergot alkaloids

Clavicipitaceae

Claviceps purpurea

Ergotism (convulsive/ gangrenous)
Rye (St Anthony's fire)

Epichloë/ Neotyphodium

LolitremB (neurotoxin)
Ryegrass (staggers in sheep)

Peramine
Insect feeding deterrent

***Endophyte**: Bacterium of fungus living inside plants without causing harm to the plant.*
Thus NOT a phytopathogen!
Rhizobium & Mycorrhiza often excluded from this definition

Endophytes – Microbial life inside plants!

Gibberella Ear Rot caused by *Fusarium graminearum*

- DON mycotoxin accumulation in Maize

Bacterial endophytes from wild maize (teosintes)

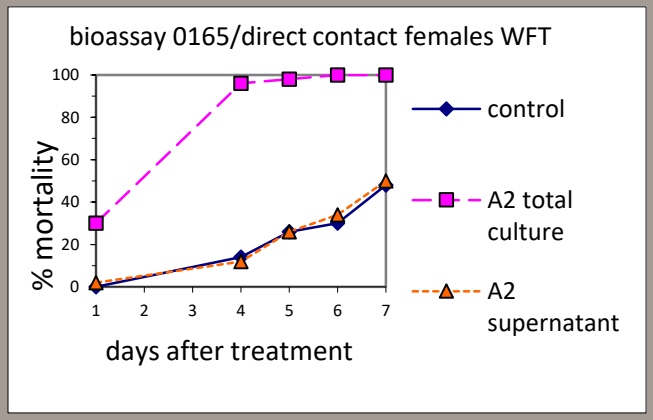
- 3 *Paenibacillus polymyxa* and one *Citrobacter* sp. strain, active compound in *P. polymyxa* was fusaridicin

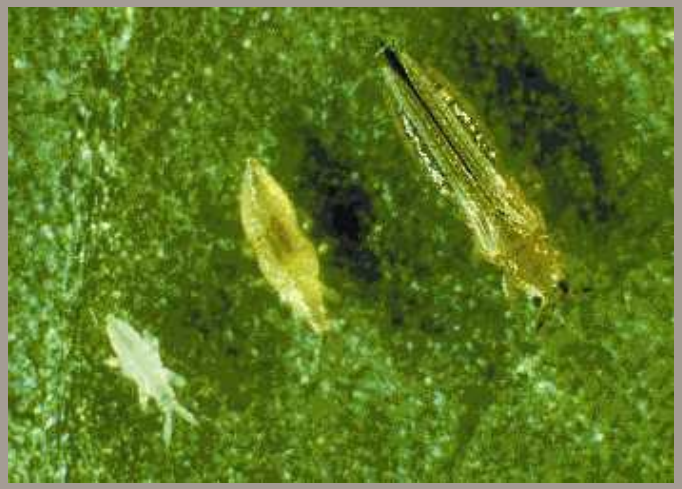
- disease suppression and food safety
- antagonizing microbes in old varieties

Mousa et al., 2015, Frontiers Plant Sci.
doi: 10.3389/fpls.2015.00805

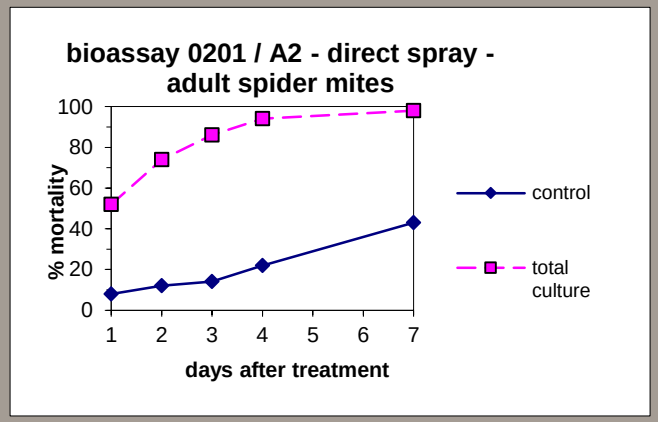
Serratia marcescens A2 against thrips and mites

Frankliniella occidentalis





Tetranychus urticae



Introduction to the plant microbiome

- There is a microbial balance in plants; the plant microbiome

  - What can happen when balances become disturbed?
  - Is it possible to restore this balance by addition of 'beneficial' microbial and, or by chemical additives to plants?
  - Is it possible to manipulate the microbiome is such a way that it becomes beneficial to plant health?

Key questions

- What is a microbiome?
- What is the microbiome of a plant?
- How to analyse plant microbiomes?
- How to manipulate the plant microbiome?

Definition…?

Microbiota

Metagenome

Microflora

..the micro-organisms in a particular environment..

.. the combined genetic material of the microorganisms in a particular environment..

.. collective genome of all organisms in a particular environment..

.. collective genome of indigenous microbes..

'A characteristic microbial community occupying a reasonably well defined habitat which has distinct physico-chemical properties'

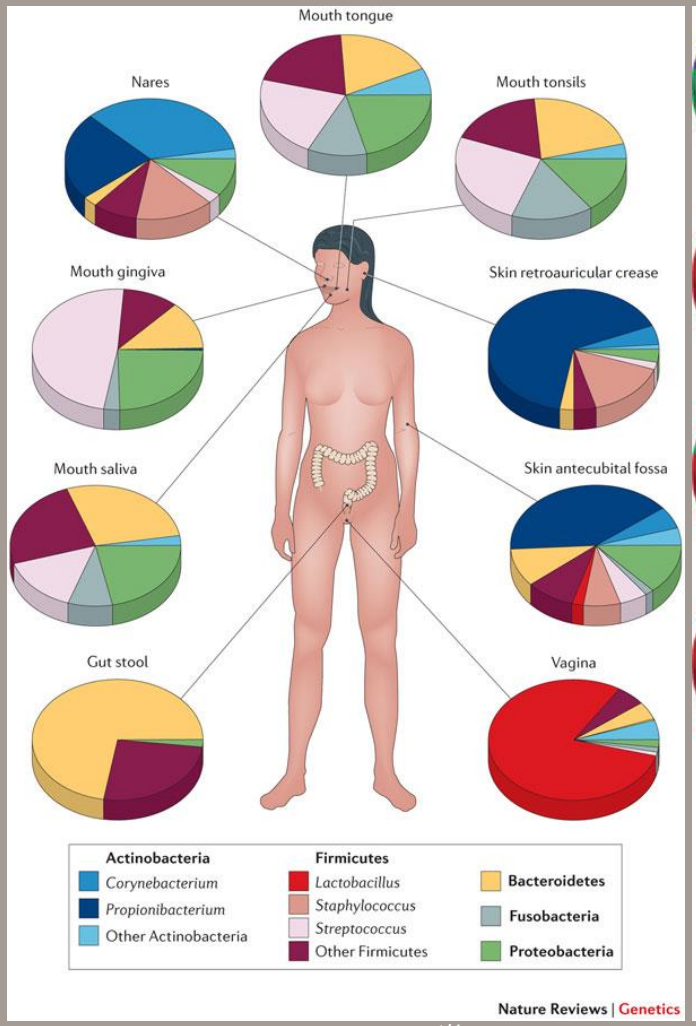
(John Wipps, 1998)

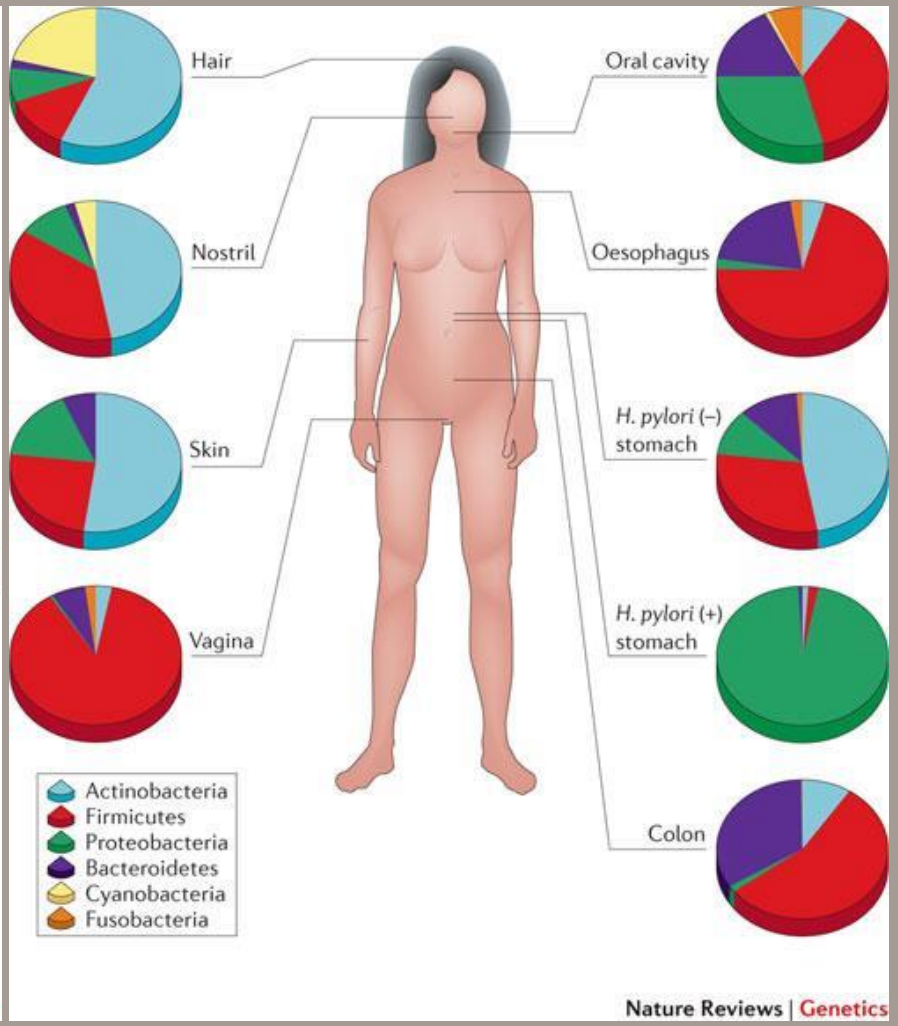
16/18S rRNA

Microbial Community

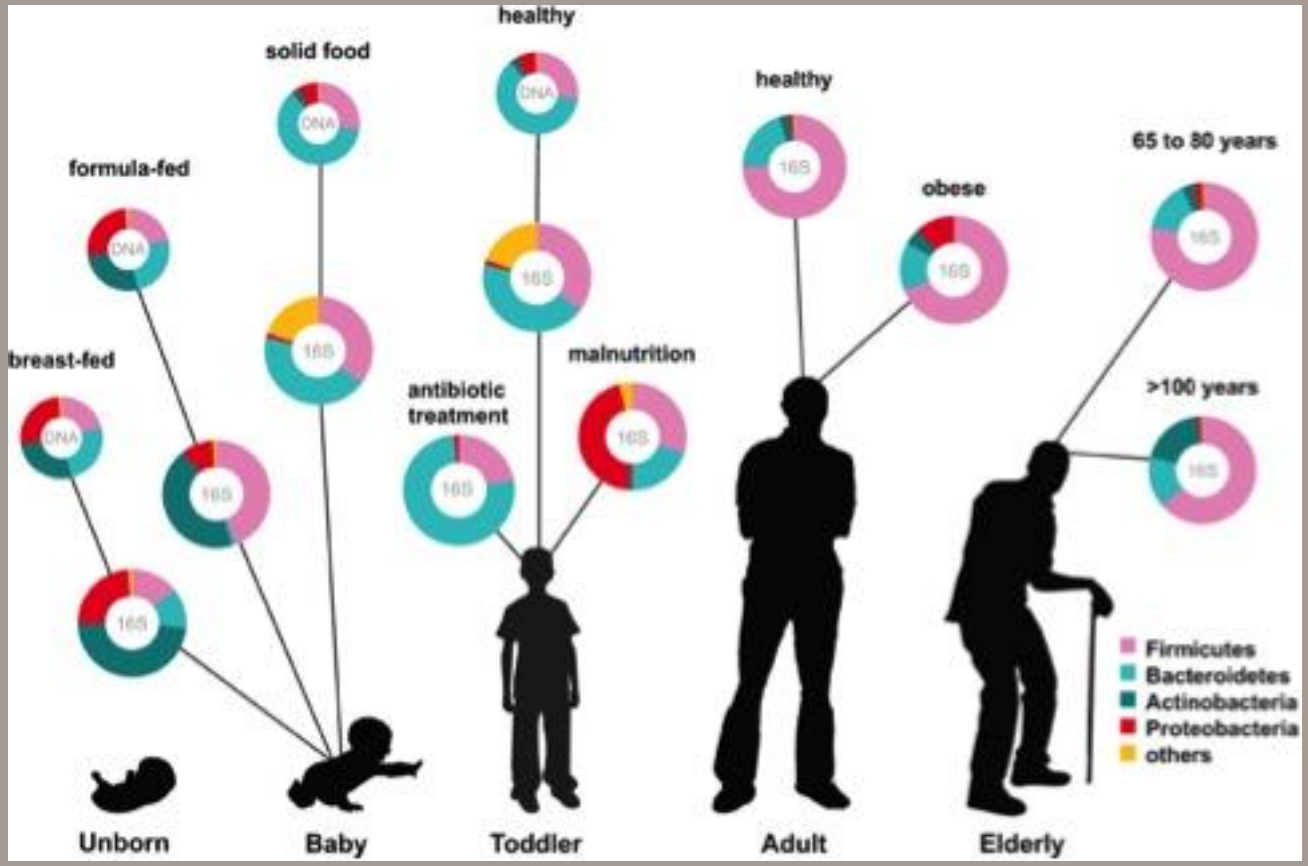
Next Generation Sequencing

The human microbiome

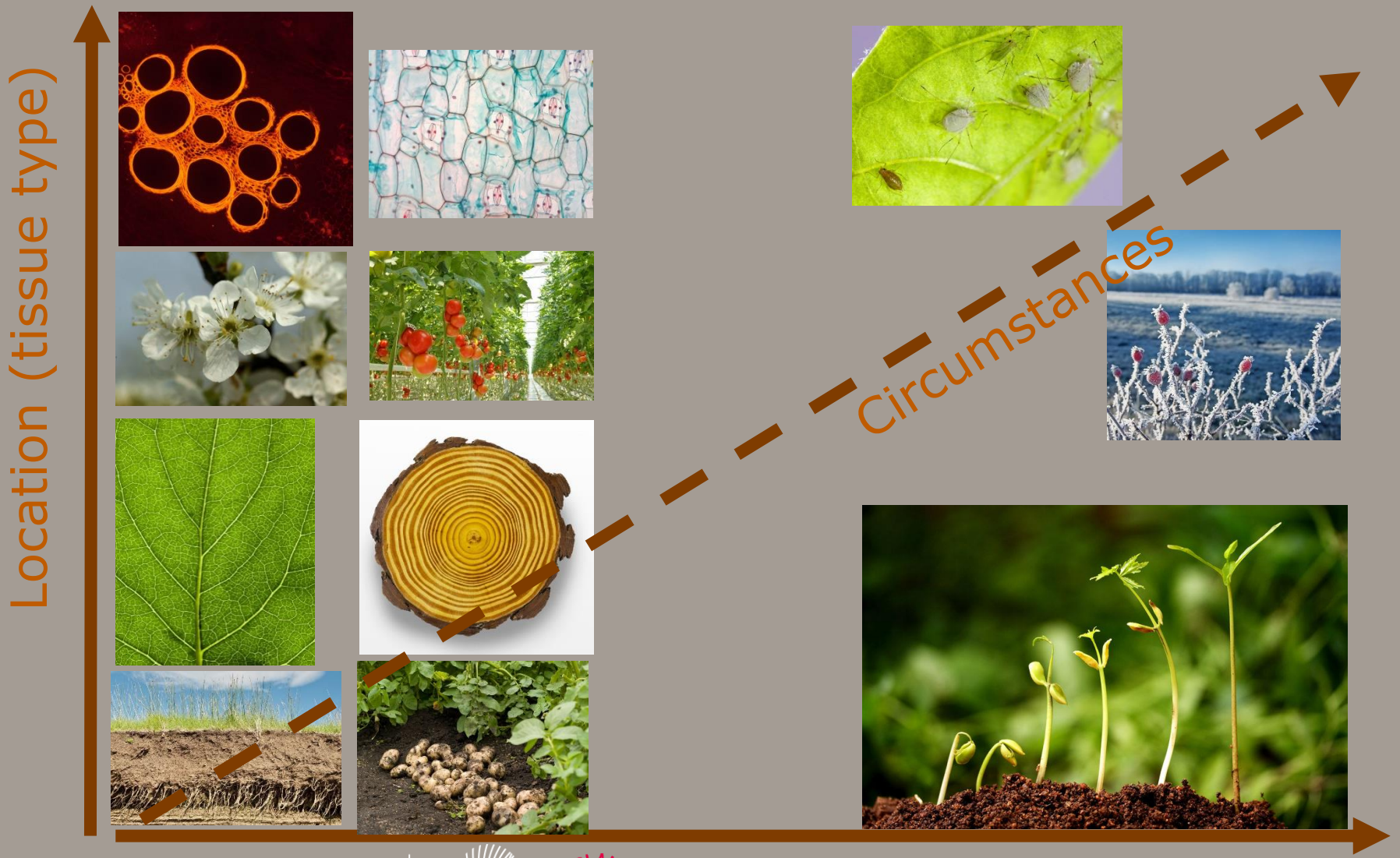




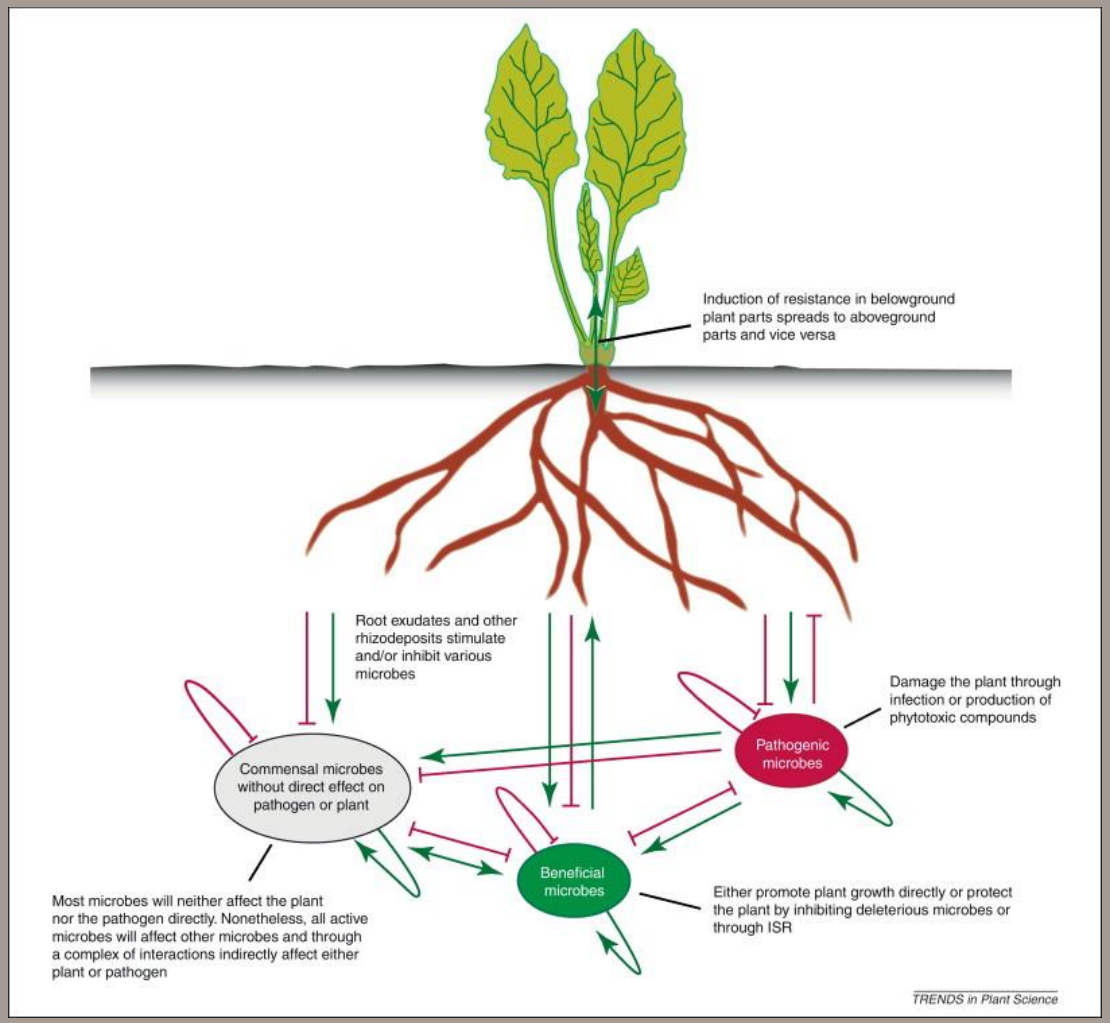
Dynamics in human microbiome composition



Plant microbiome composition dynamics



Microbial functions in plants



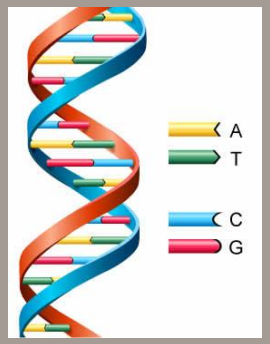
Plant Beneficials: 'the good'

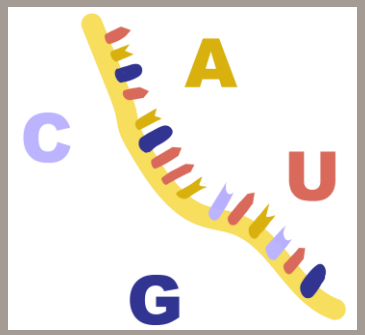
Plant Pathogens: 'the bad'

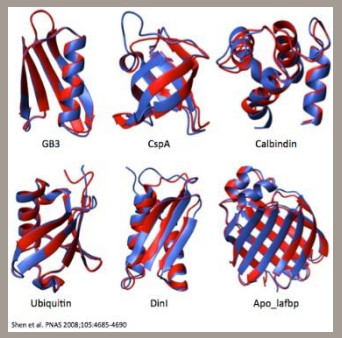
Human Pathogens 'the ugly'

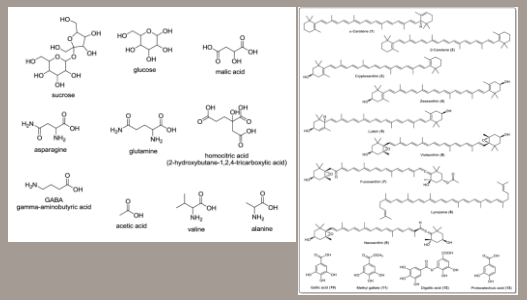
Omics technologies – untargeted approaches-

DNA	RNA	Proteins	Metabolites









Metagenomics

Species composition
Microbial functions
Metabolic pathways

RNAseq/ metatranscriptomics

Gene expression profiling
Cellular processes
Microbial functions

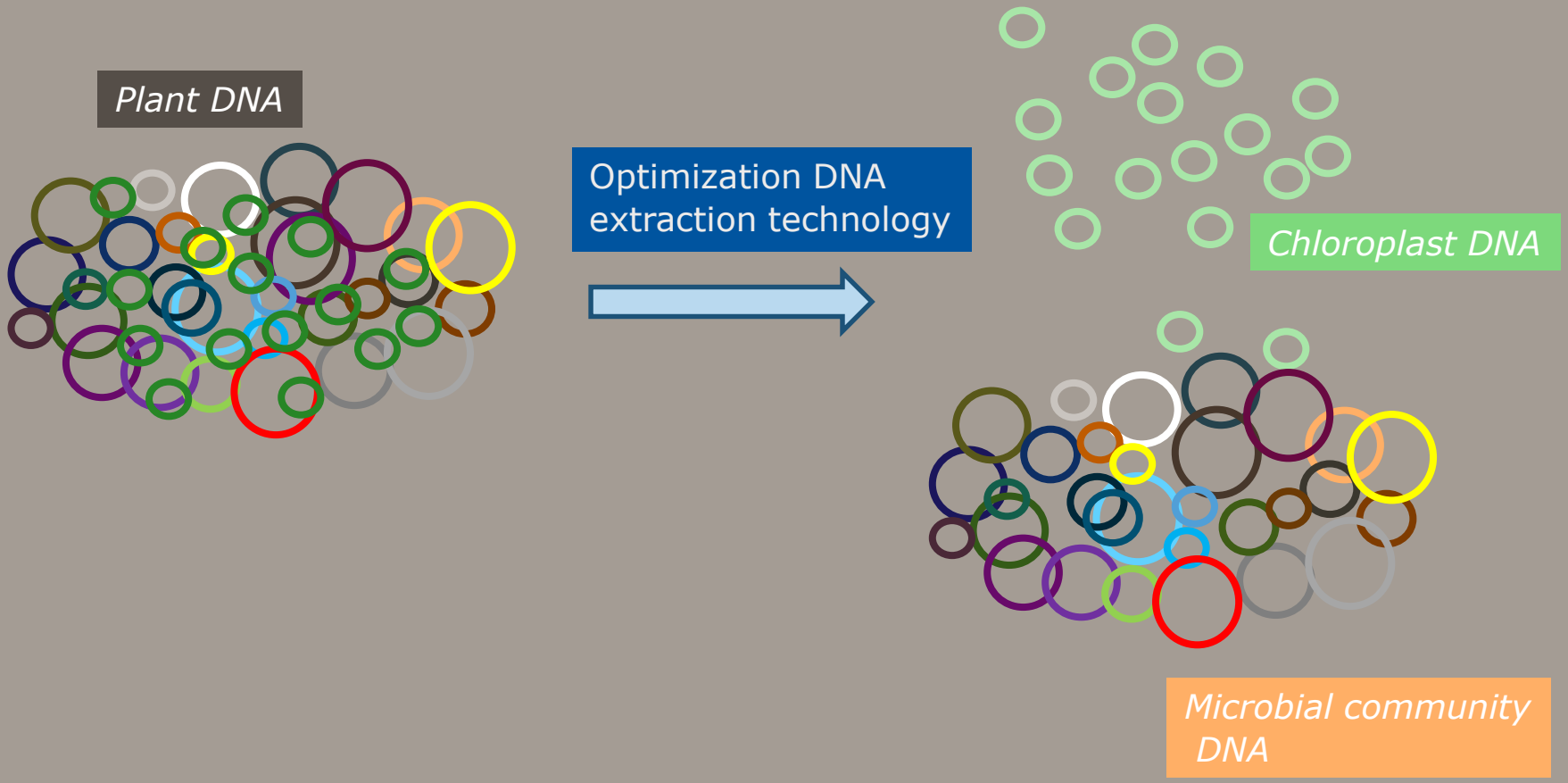
Proteomics

Gene expression & Translation
Gene expression
Cellular processes

Metabolomics

Primary & secondary compounds
Gene expression
Cellular processes

Chloroplast (cell organelle) separation from Plant DNA



Next Generation Sequencing

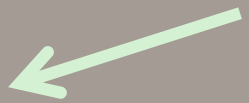
Next Generation Sequencing Platforms

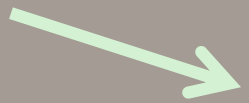
Life Sciences (Roche) 454 (2004) ,

Illumina Miseq/ Hiseq,

Thermo Fisher Ion torrent

Pacific Biosciences (Roche) PacBio





Fragment sizes

150 bp (454)
> 20 kb (PacBio)

accuracy

0.1 – 1%

Culture-omics

- Cultivation of novel (unexplored) microbes
- Novel cultivation steps and circumstances
- Genomics and experimentation

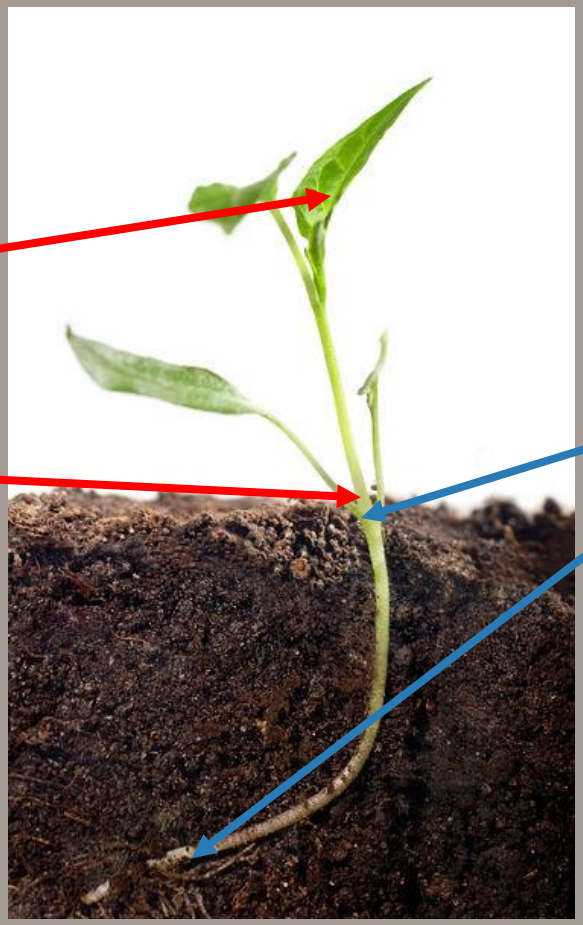
Omics applications in plant microbiology

Plant

Microbiome

Metabolomics

RNAseq

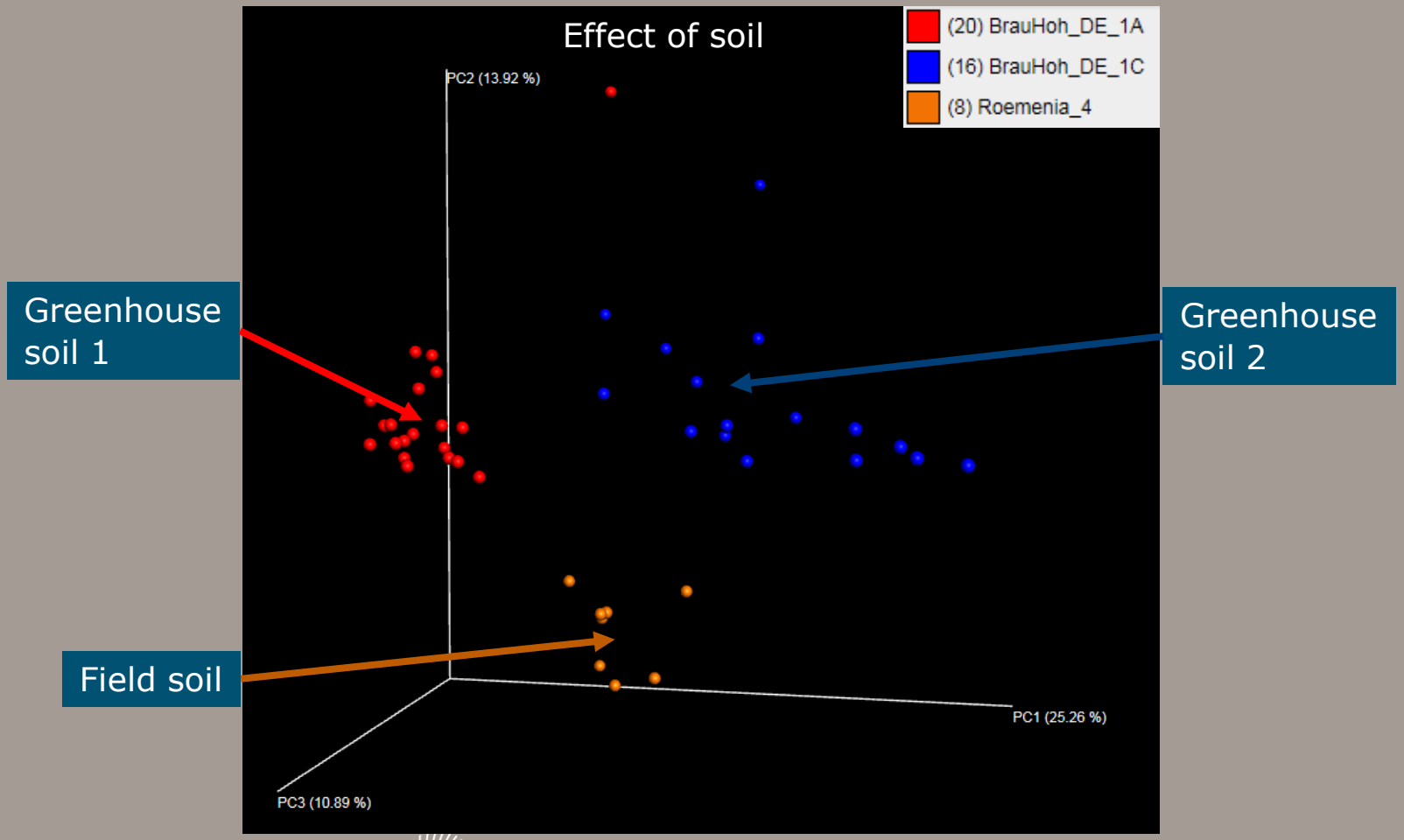


Metagenomics/
Metatranscriptomics

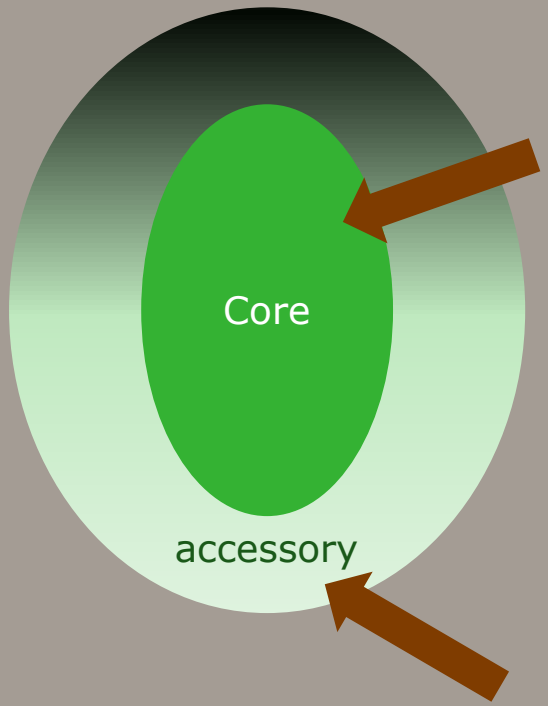
How does the plant feel?

How is the microbiome responding?

Impact on tomato plant microbiome composition by amplicon sequencing



The plant microbiome concept



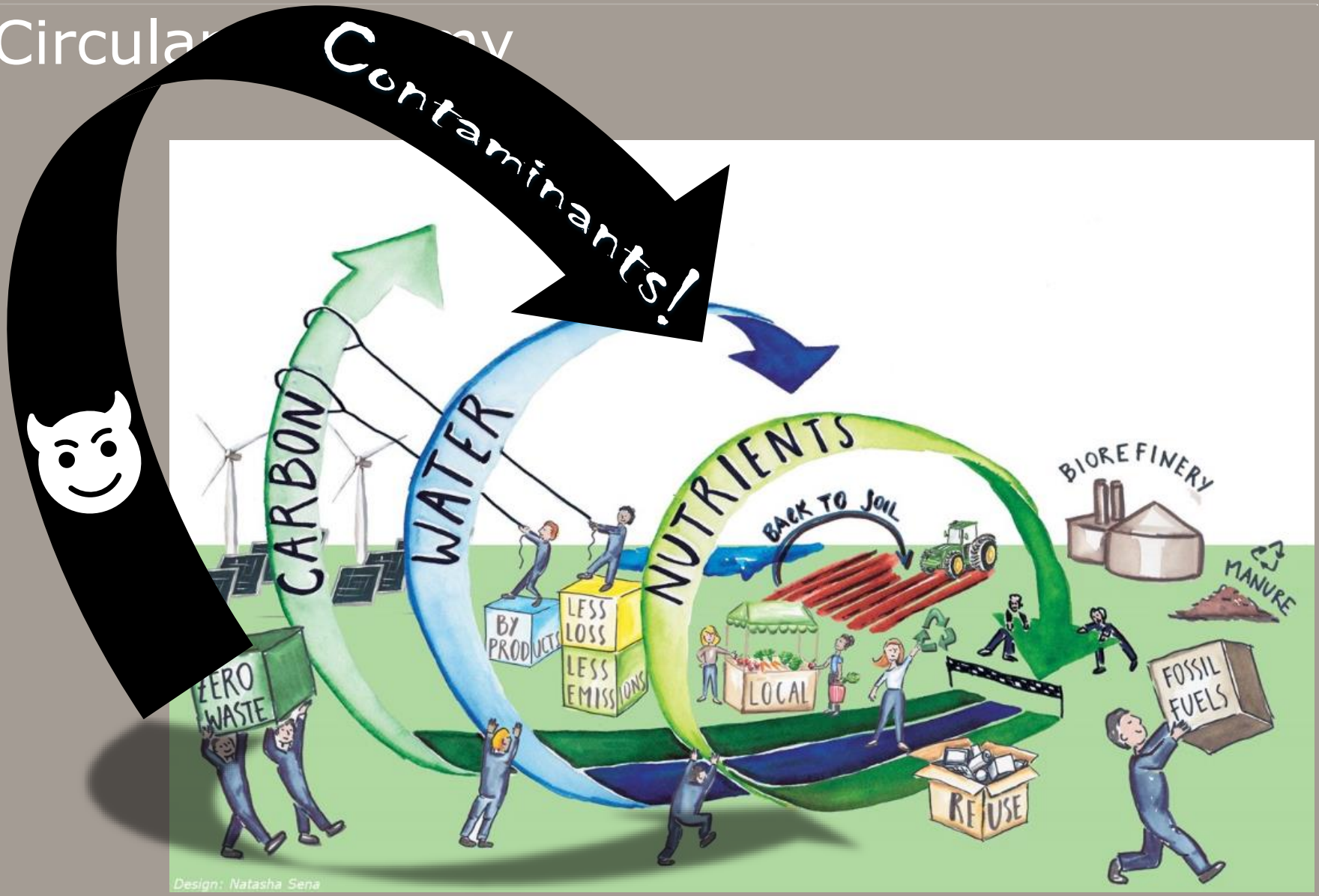
Core microbiome
Permanent present in plants over many generations

- Seed transmissible
- Obligate endophytes
- Required for plant growth & development
- Plant (endo) symbionts
- Single habitat (small genome size)

Accessory microbiome
Not always present

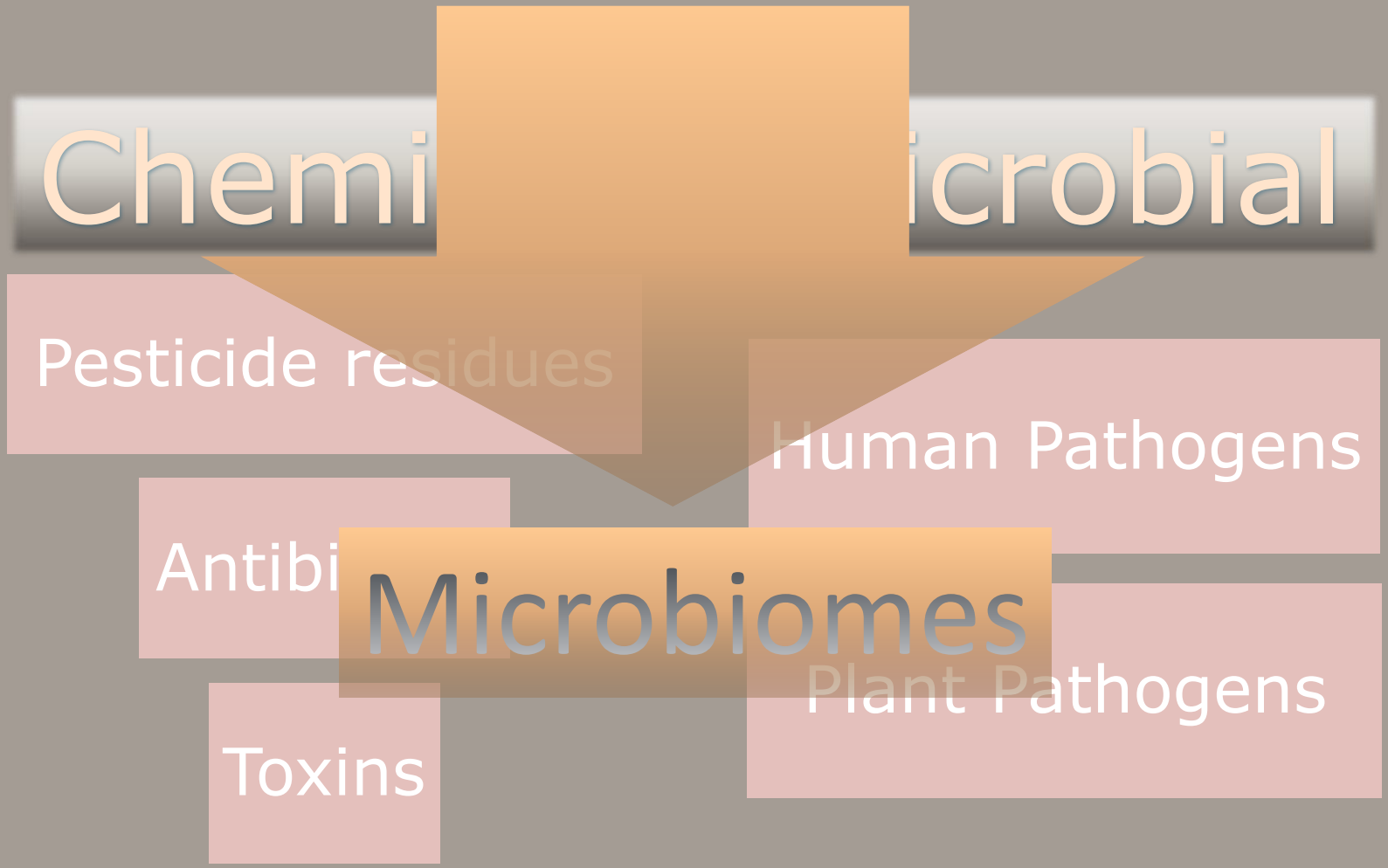
- Always acquired (Environment)
- Required for plant growth in context dependent way
- Horizontally transmitted
- Multiple habitats (large genome size)

Circular [illegible]y

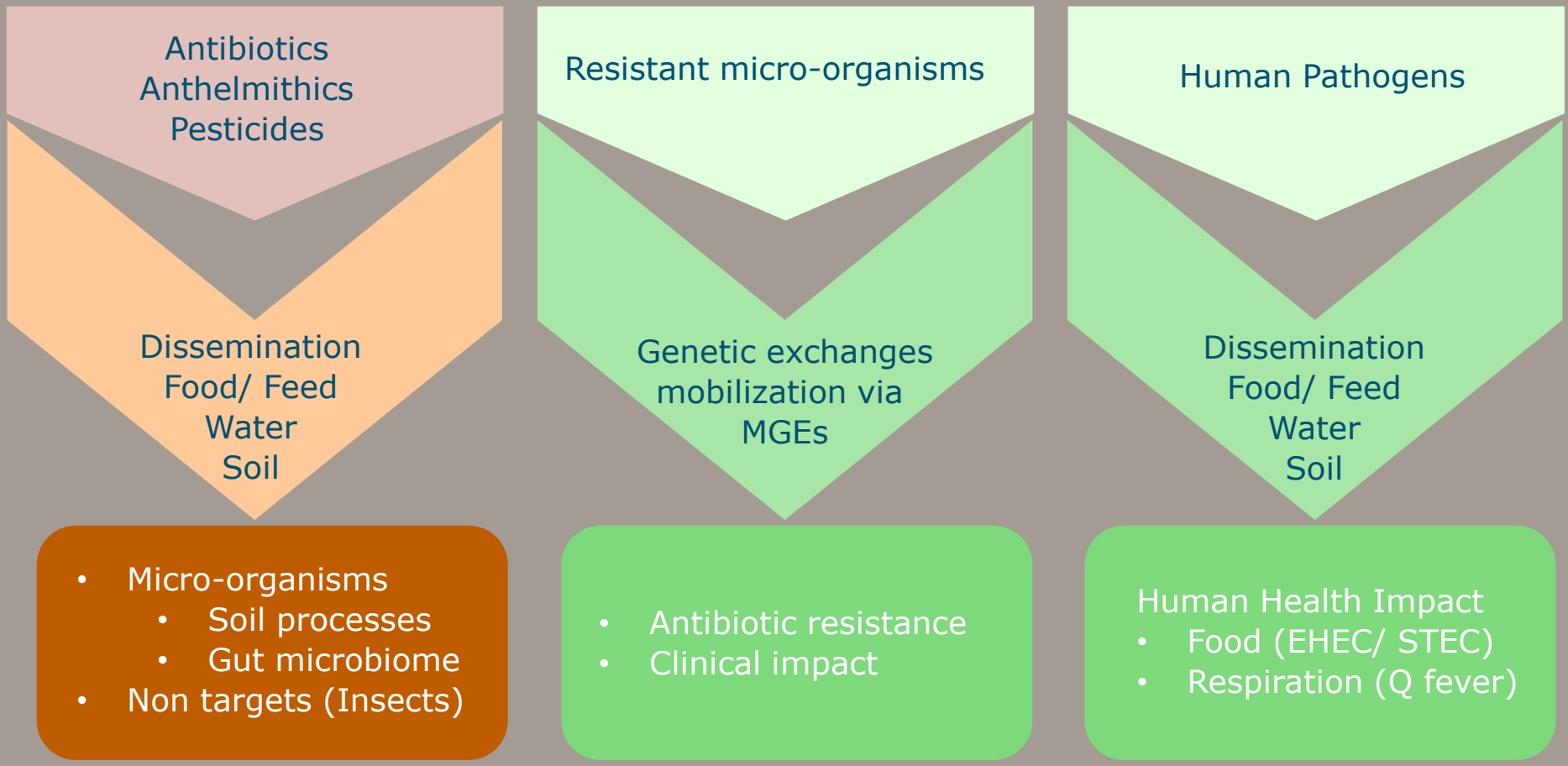

Contaminants!
CARBON
WATER
NUTRIENTS
BACK TO SOIL
BIOREFINERY
MANURE
BY PRODUCTS
LESS LOSS
LESS EMISSIONS
ZERO WASTE
LOCAL
FOSSIL FUELS
RE USE
Design: Natasha Sena

Interaction animal – plant production

Circulating contaminants



Consequences



Circulation of antibiotics

Antibiotic resistance gene association with mobile genetic elements

colistin

Polymyxins

mcr family → Inc FII, H, HI1, X4, I2 plasmids

mcr-1 → Inc P-1

norfloxacin

(Fluoro) quinolones

qnr family → Inc A/C, H12, F, FII, L/M, N, R, U, I1, ColE1 plasmids

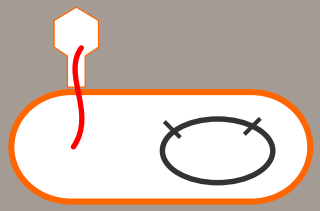
qnrS2 → Inc Q

Shewanella algae, S. putrefaciens
Predominating in aquatic environments
(Marine & Fresh water, sewage)

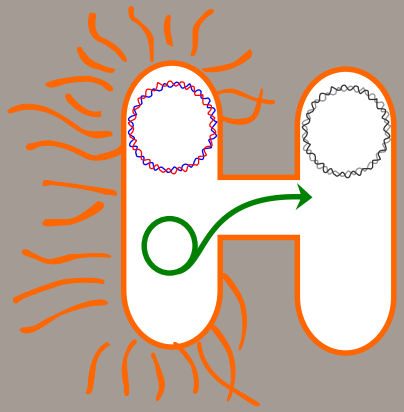
Lateral transmission of antibiotic resistance genes

Antibiotc selective pressure

Transformation



Transduction



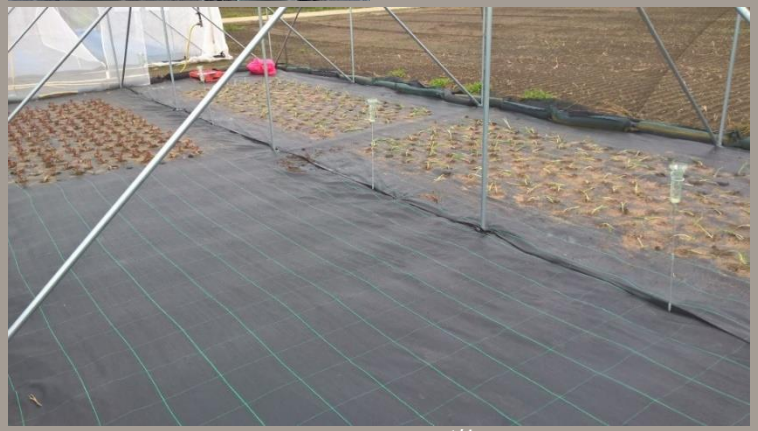
Conjugation

One health in plant production

Field experiment with manure application to plants



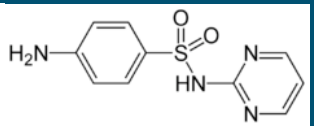




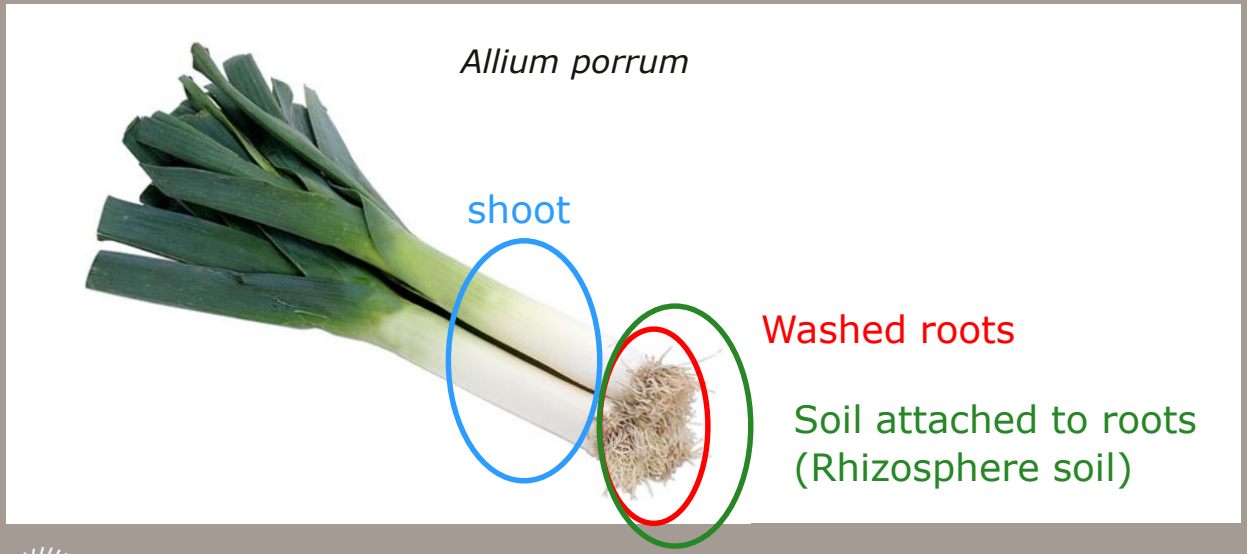

Sulfadiazine (Sf) resistance in leek plants

‘ Mobile’ antibiotic Sulfadiazine added to cattle manure (10 ppm):
‘ Early’ : 37 days before planting to leek
‘Late’ : 2 days before planting to leek

Sufadiazine (Sf); mobile antibiotic

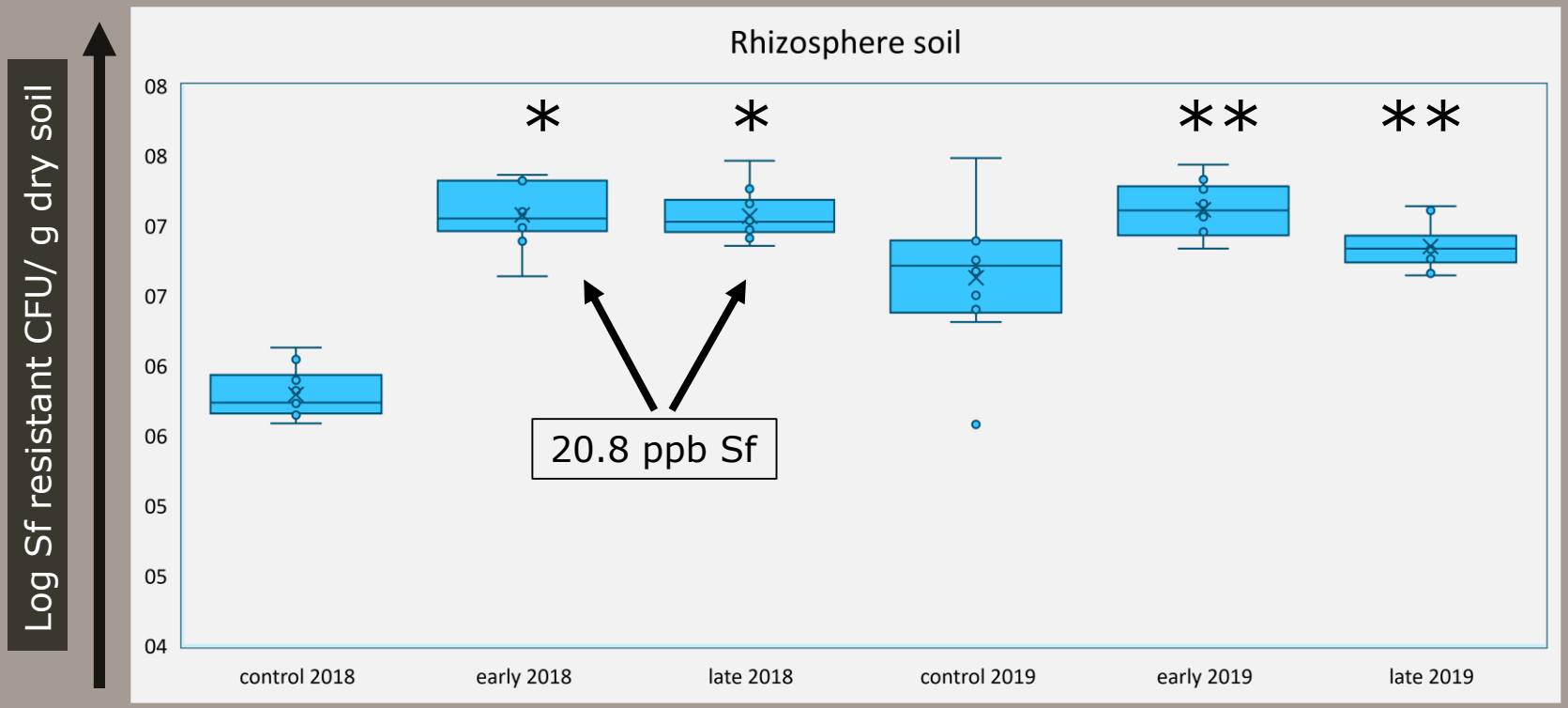


At planting 82 ppm Sf in ‘early’ manure and 11 ppm in ‘late’ manure



Sulfadiazine resistant bacteria in leek rhizophere

* and **; significantly different to respective controls, i.e. * for 2018 and ** for 2019



August 2018 — Planting

November 2018 — 1st harvest

May 2018 — 2nd harvest

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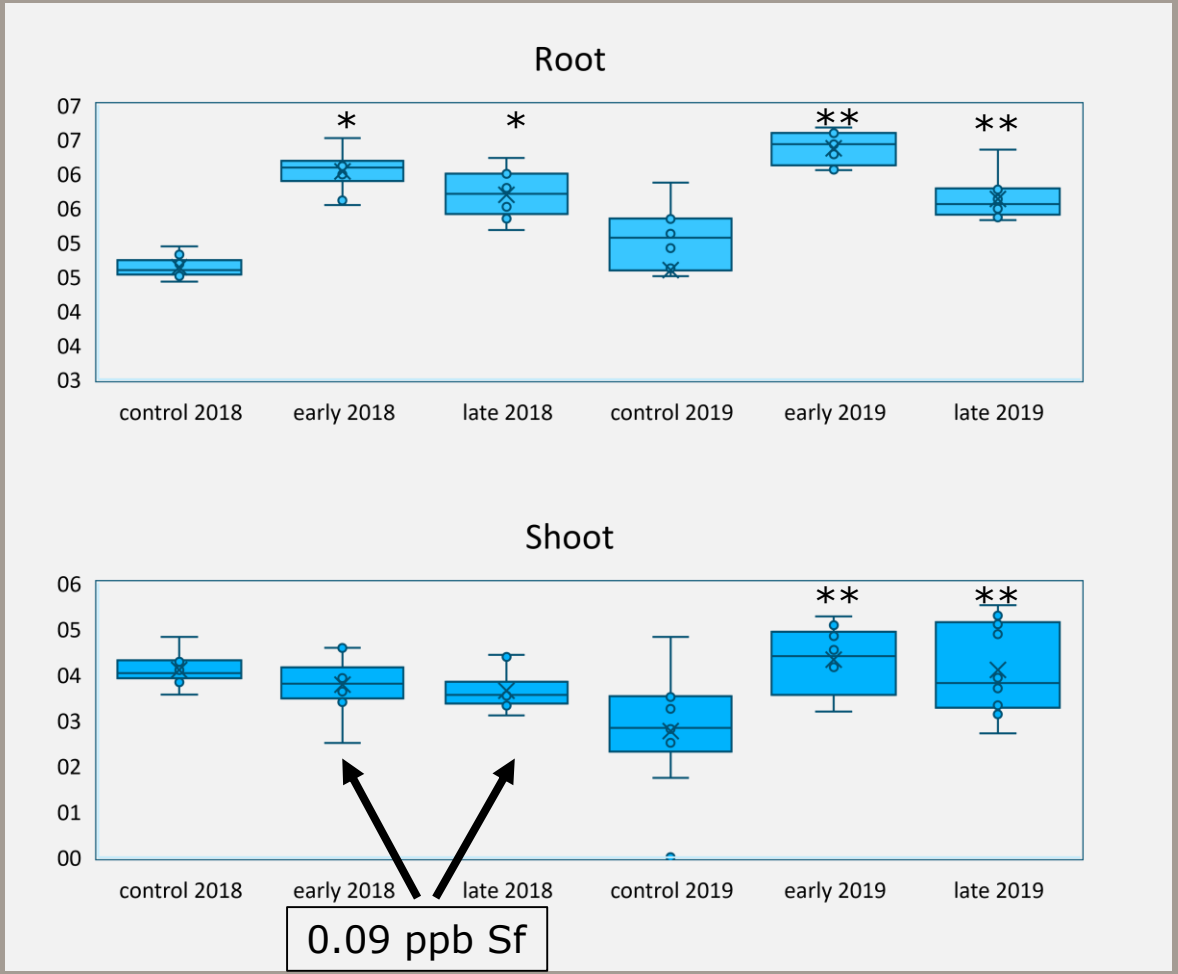
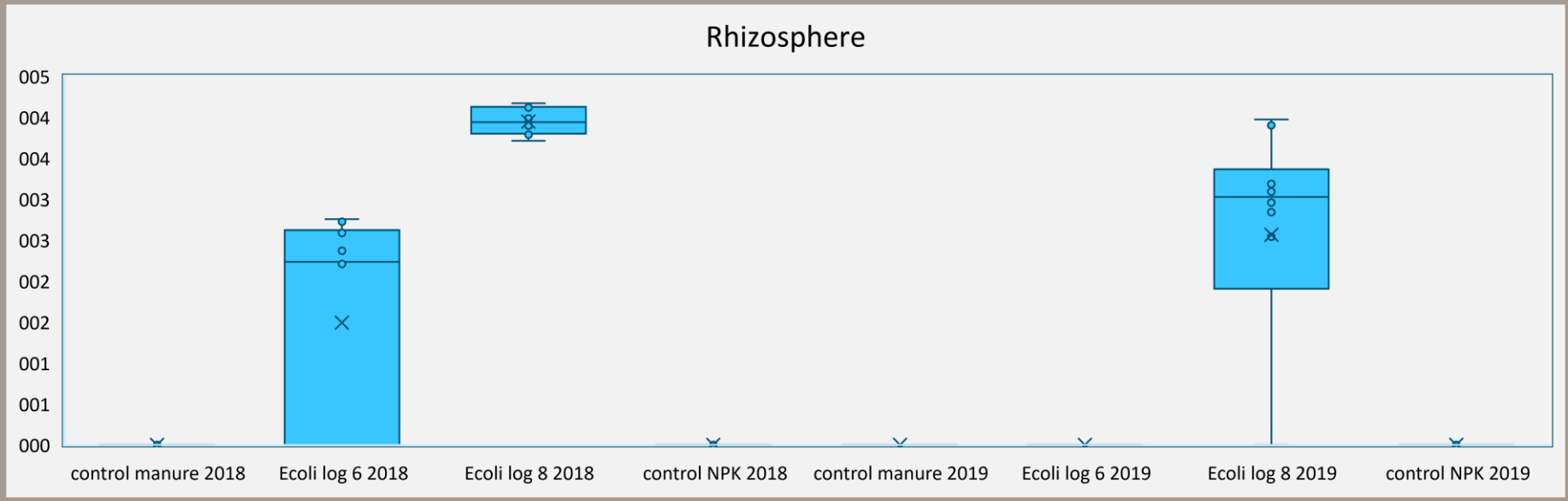
Sf-resistant bacteria in leek plants



E. coli in leek rhizosphere soil



2018, 3 months after planting

2019, 9 months after planting

Conclusions field experiment with manure

- Sulfadiazine was taken up by lettuce and leek plants in low quantities.
- Higher Sf-resistant bacterial numbers were found in the rhizosphere and roots of lettuce and leek

- *E. coli* was transmitted from manure to lettuce and leek plants and could persist in the leek rhizosphere during winter time.


Thank you for
your attention!