Deciphering the microbiomes of disease suppressive soils

Adam Ossowicki

Soil - environment rich in microbes

How many microbes ?

$4x10^3$ to $5x10^4$ species per gram

$1x10^8$ to $1x10^9$ bacteria cells

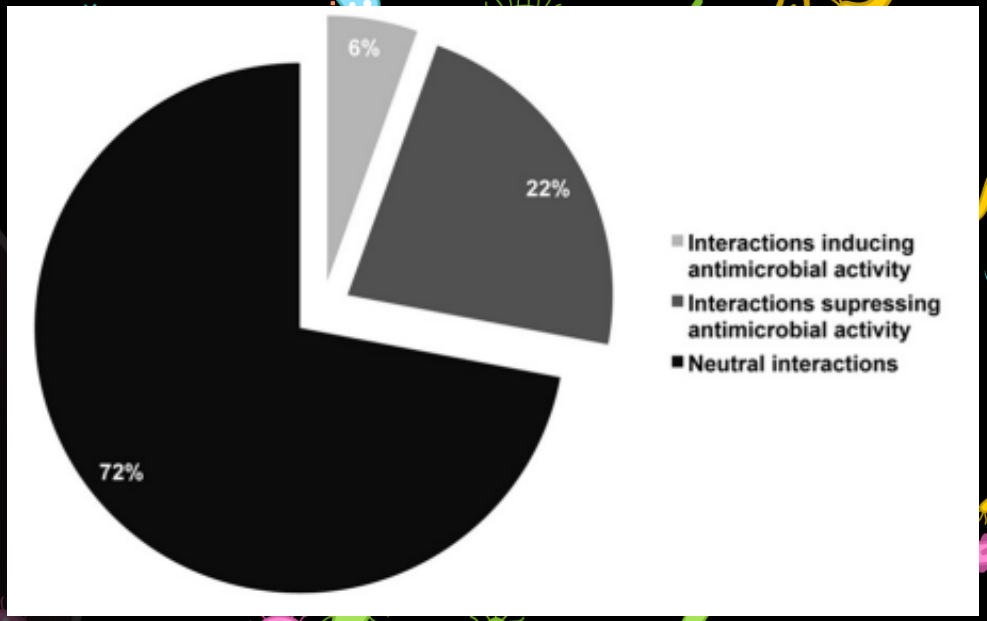
30,000 formally named bacteria species (NCBI, 2022)

Bates et al. ISME 2012 Nunan N., PLoS One 2014

Soil - interactions

Single bacteria cell can potentially interact with ~100 other cells

Tyc at el. tested 146 soil bacteria isolates in monocultures and pair-wise combinations (2798) for antibacterial activity against two human pathogens



Nunan N., PLoS One 2014, Tyc et al. Front. Microbiol.

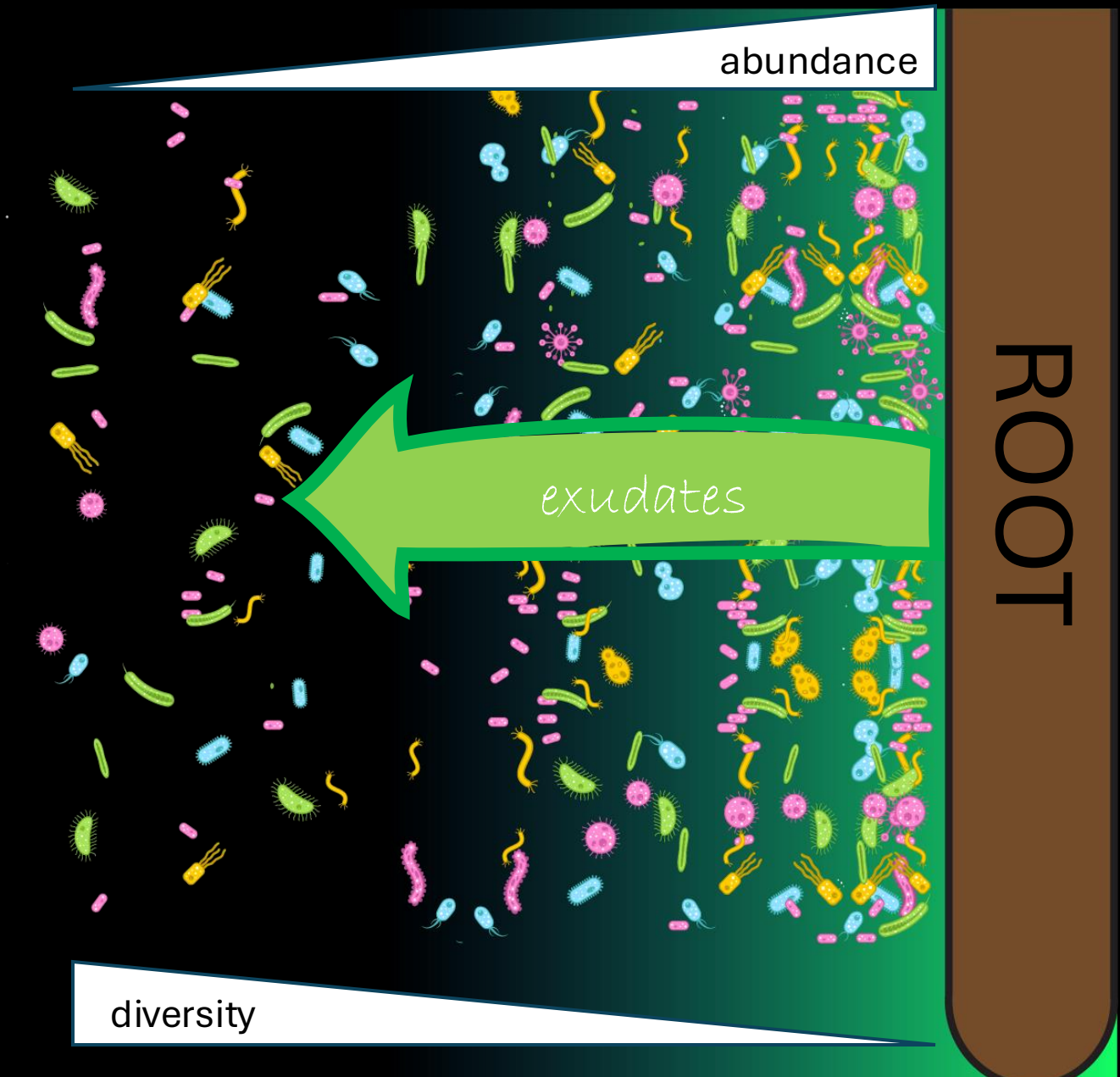
Soil microbiomes and plants

Plants allocate up to

30%

of fixed carbon into root exudates

...and they have a good reason to do that



Soil microbiomes and plants - MAPs

MAP – Microbiome-Associated Phenotype (Oysterman et al. COMICR 2018)

Stress tolerance

Plant growth promotion

Nutrient cycling

Pest control

Degradation of pollutants

Climate regulation
(e.g. greenhouse gas mitigation)

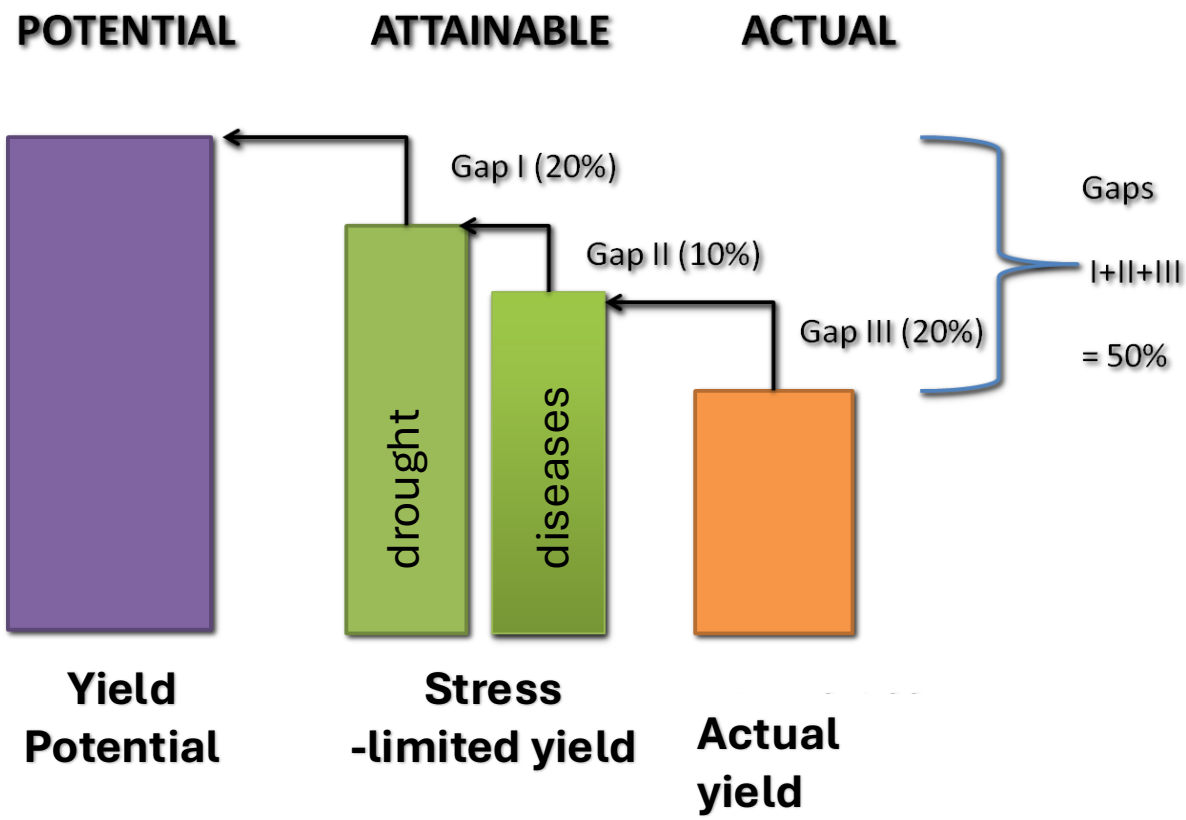
Abiotic
- Heat
- Cold
- Drought
- Flooding

Biotic

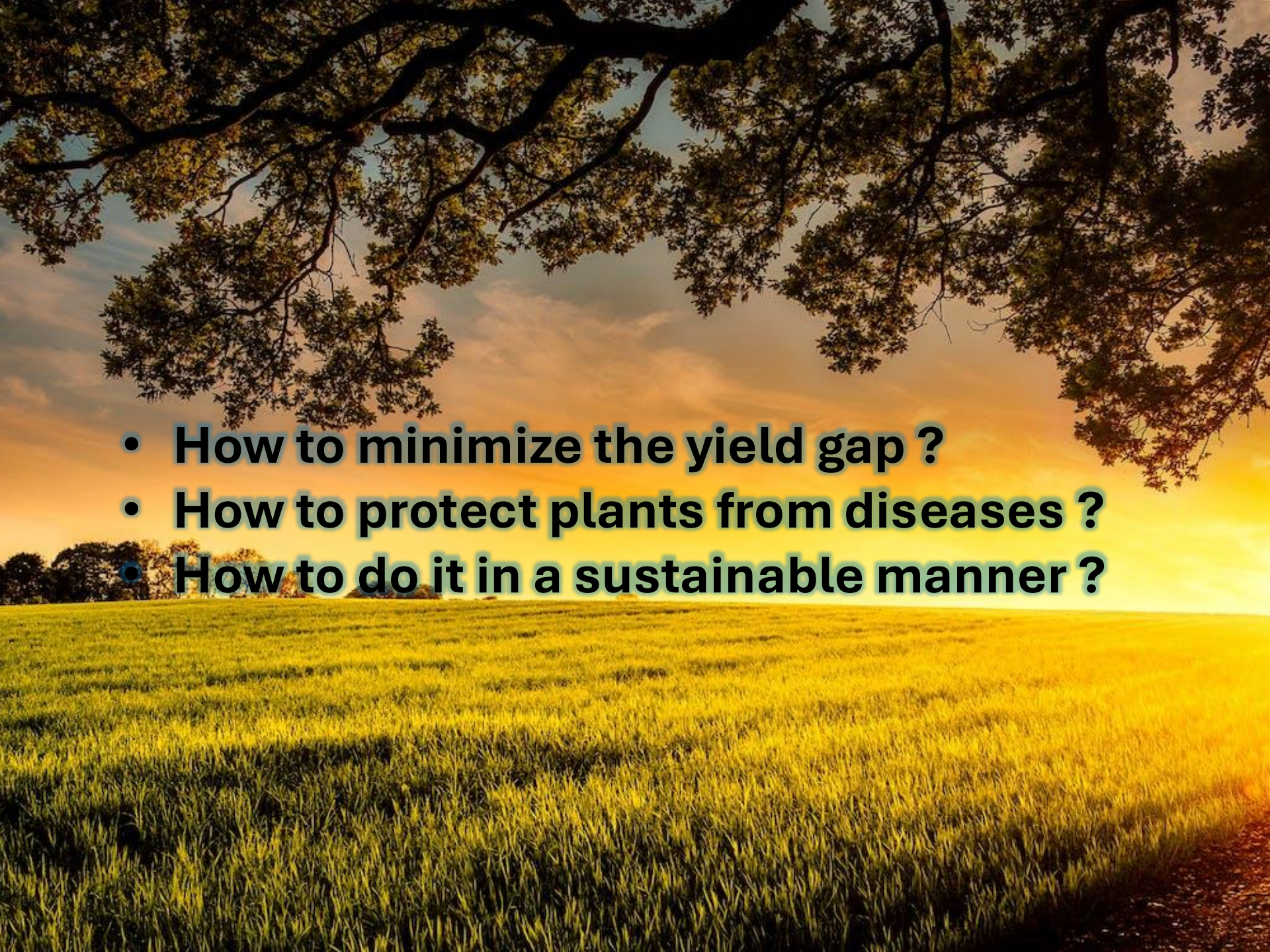
Pathogens

Hartmann, M. and Six, J. *Nat Rev Earth Environ* 2023, de Vrieze, J., Science 2015

Yield Gap

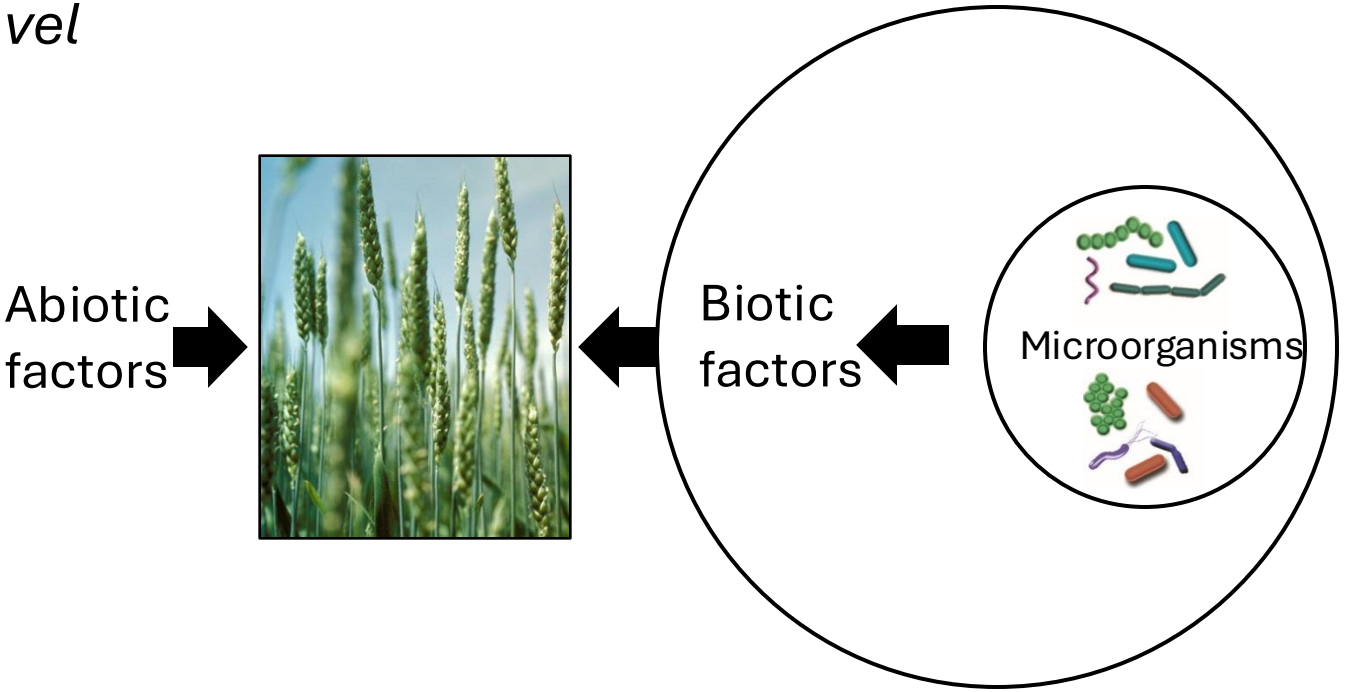


MODAGS project , www.mtt.fi, modified


• How to minimize the yield gap ?
• How to protect plants from diseases ?
• How to do it in a sustainable manner ?

Soil disease suppressiveness **a sustainable alternative for controlling diseases**

Soil disease suppressiveness – natural phenomenon where despite presence of pathogen and favorable conditions disease occurs at a very low level



Understanding disease suppressive soils:

- *Sustainable microbiome-based crop protection*
- *Biological alternatives to chemical pesticides*
- *Soil health preservation / restoration*

Soil disease suppressiveness around the world (selected)

Fusarium oxysporum in vegetables and fruits
France
Korea
Hainan island China

Take-all disease in wheat
Washington State, US
The Netherlands
Montana, US
Chile

Rhizoctonia solani AG2 in sugar beet
The Netherlands

Rhizoctonia solani AG3 in potato
Greenland

Rhizoctonia solani AG8 in wheat
South Australia
Pacific Northwest of the USA

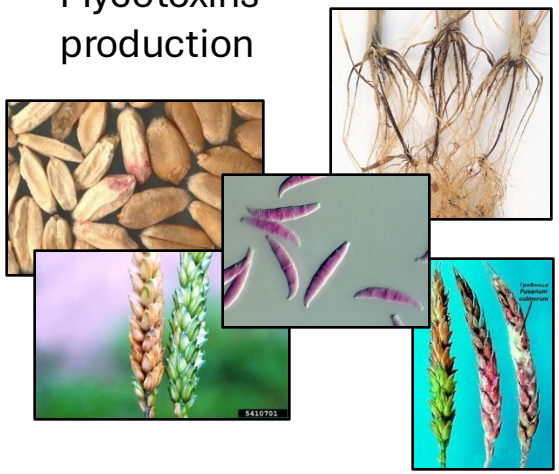
Case study: disease suppressive soil

How to find a suppressive soil ?

Pathogen

Fusarium culmorum

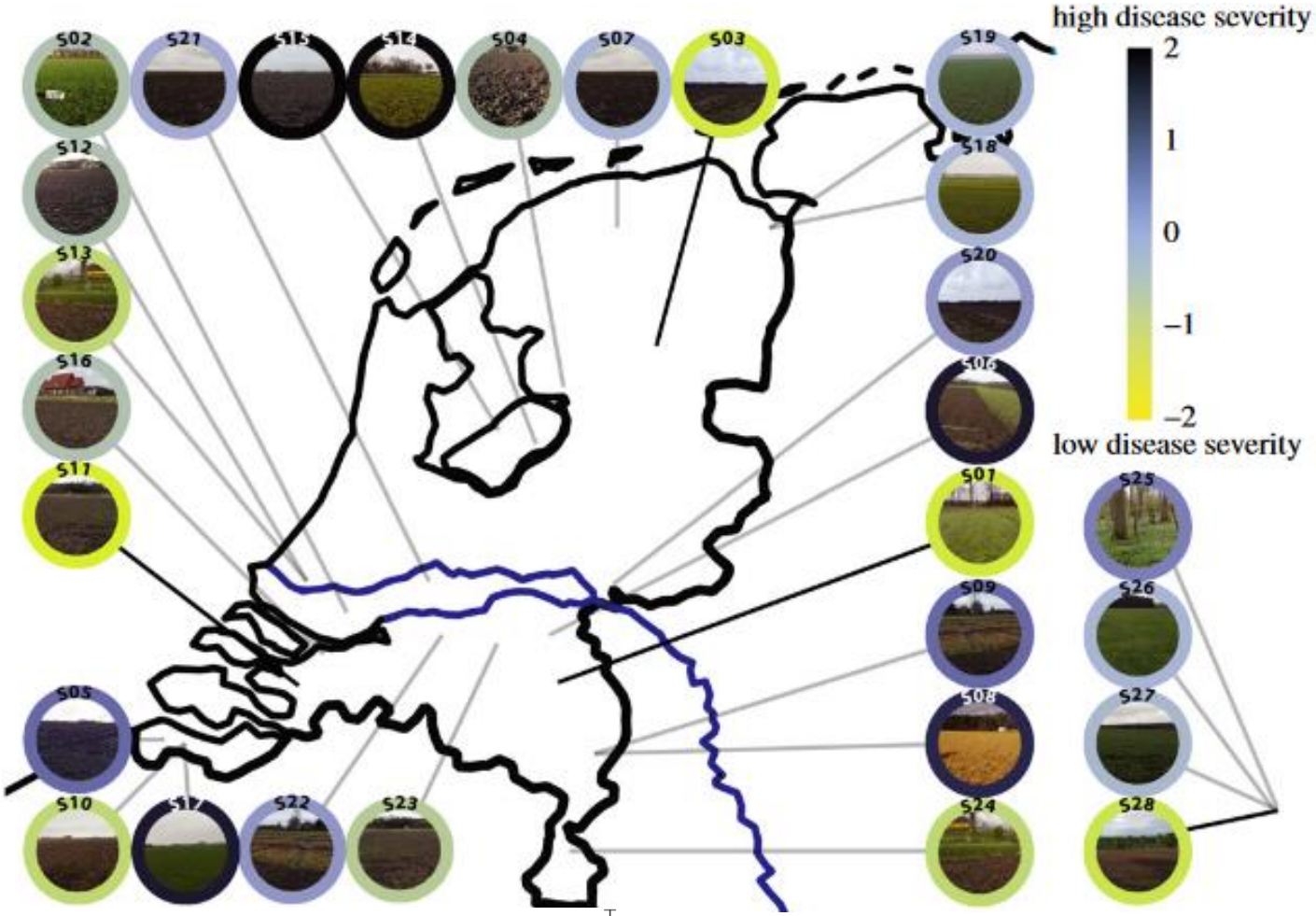
- Foot and root rot
- Head blight
- Mycotoxins production

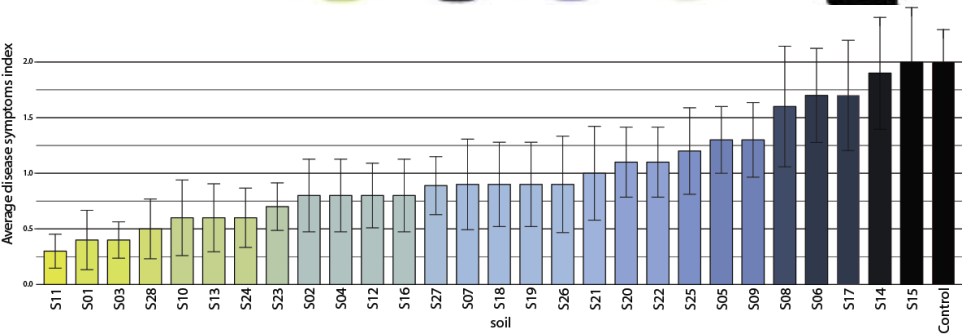


Host

Wheat - *Triticum aestivum*

Soil disease suppressiveness - profiling





Ossowicki, A. *et al. Proceedings of the Royal Society B* (2020)

What makes a soil suppressive ?

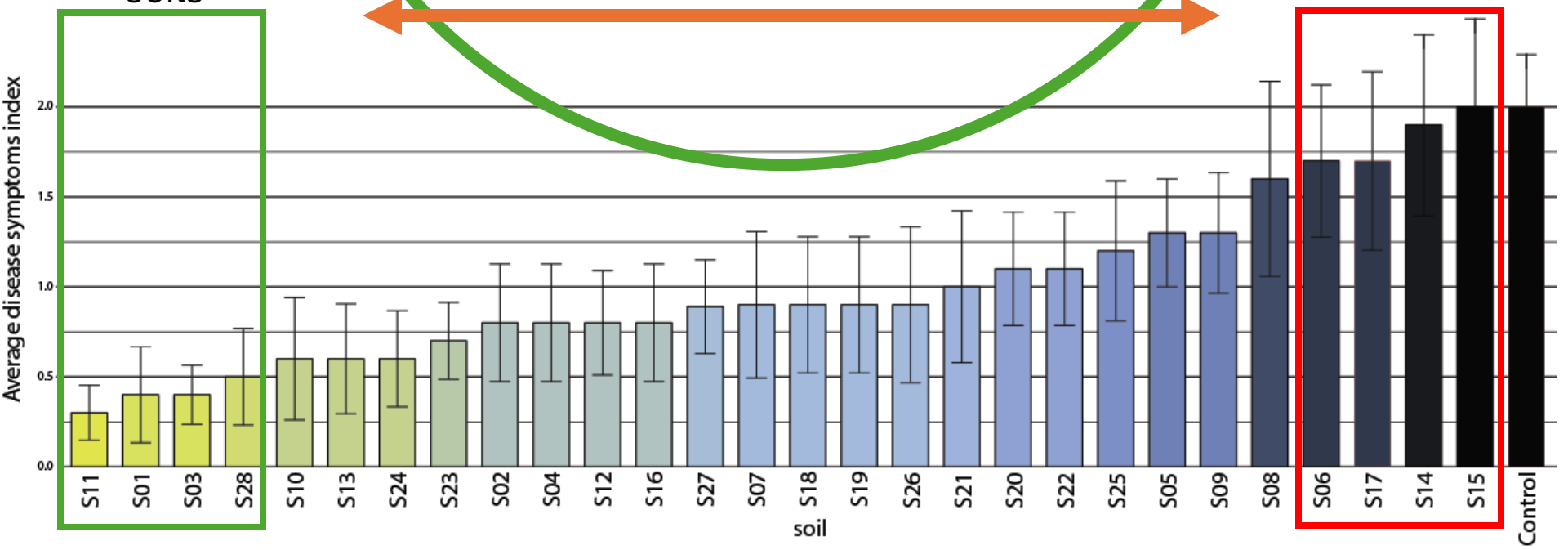
What are the key microbial taxa involved ?

What metabolites are produced ?

What are key microbial functions involved ?

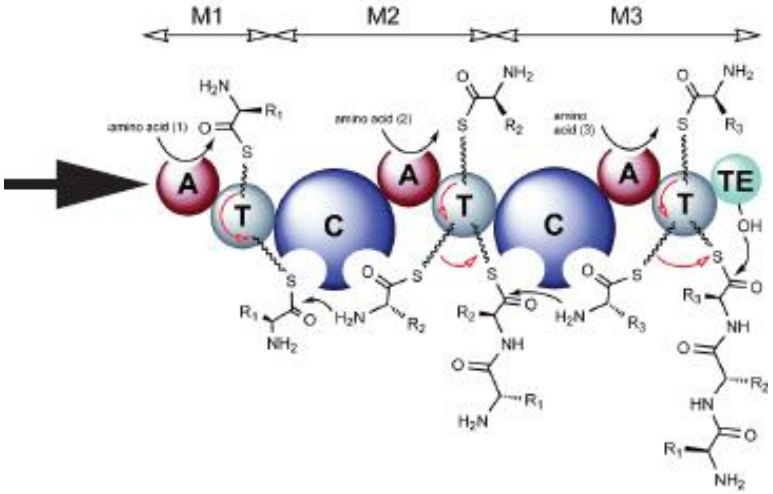
Suppressive soils

Conducive soils



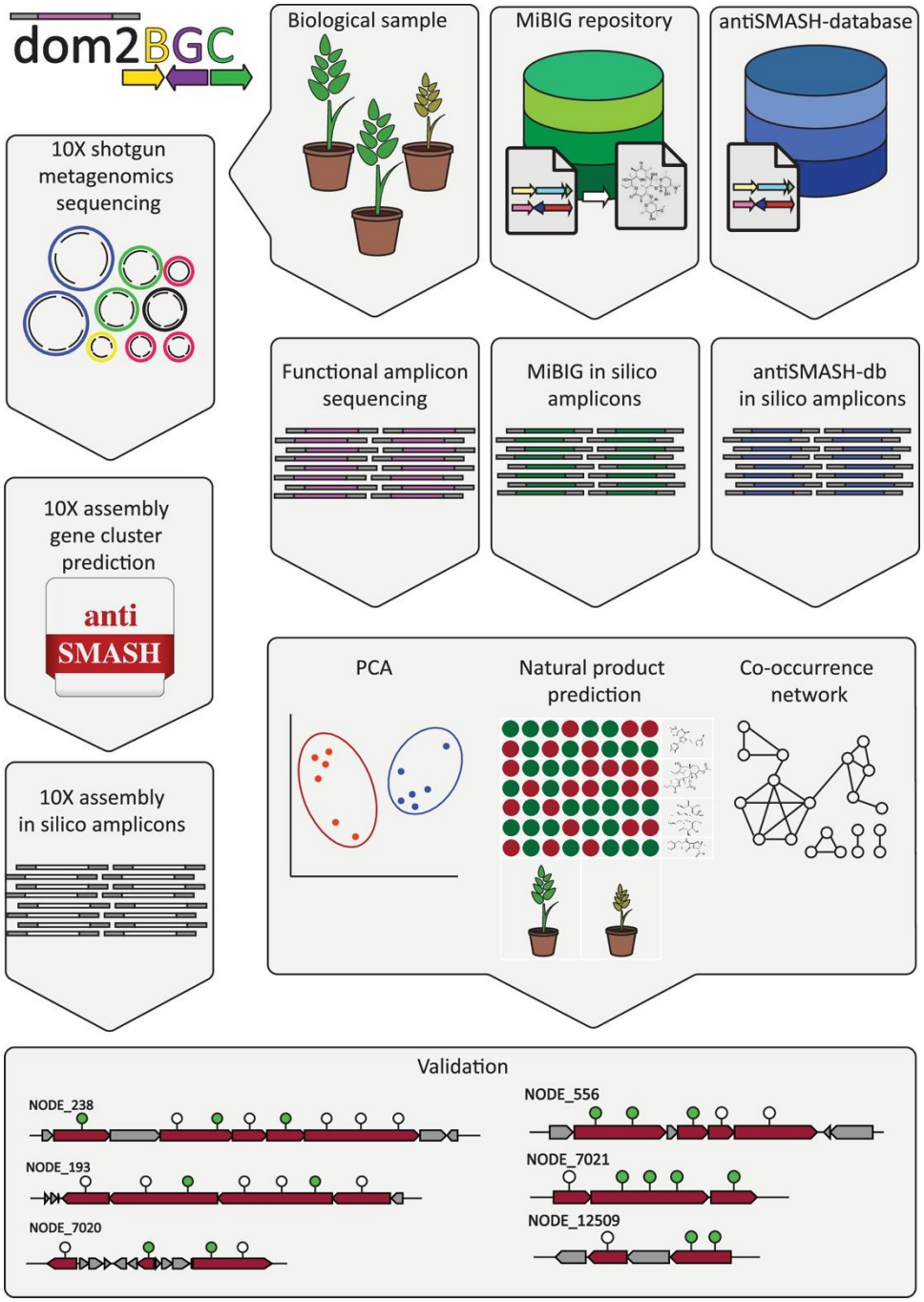
Amplicon sequencing:

16S + Adenylation domains + shotgun metagenomes



Adenylation domains are parts of biosynthetic gene clusters,
-NRPS (nonribosomal peptide synthetase)
-Hybrid NRPS-PKS clusters (NRPS-polyketide synthase)

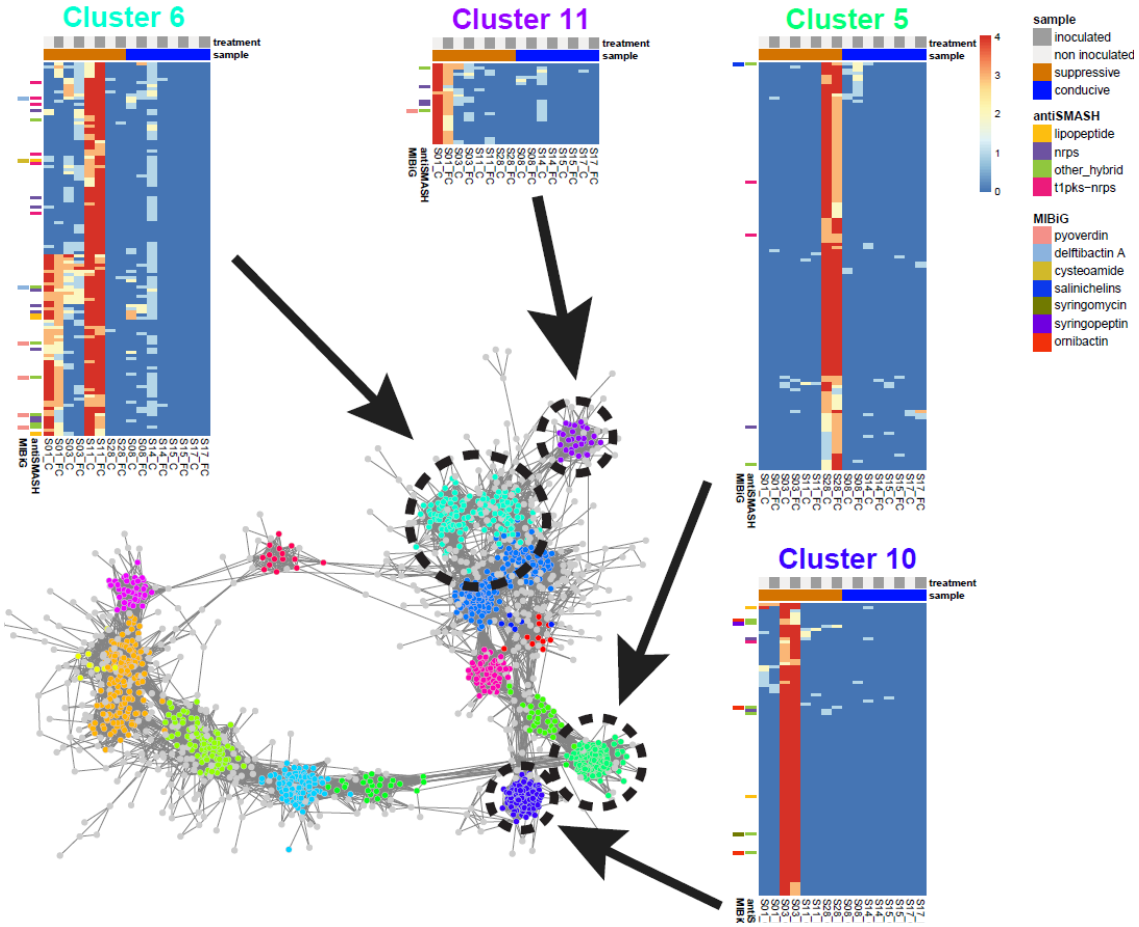
Dr. Vittorio Tracanna



dom2BGC pipeline , https://git.wur.nl/traca001/dom2bgc

Taxonomic and functional profiling

Ossowicki, A. *et al. Proceedings of the Royal Society B* (2020)
Tracanna, V. & Ossowicki, A. *et al. mSystems* (2021)



- Different suppressive soils revealed different bacterial taxonomic patterns, diversity, physical and chemical parameters
- All the suppressive soils show uniquely overrepresented bacterial guild dominated by *Acidobacteria*
- A-domains share similar community structure in suppressive soils
- A-domains analysis indicated a role of siderophores in the plant protection

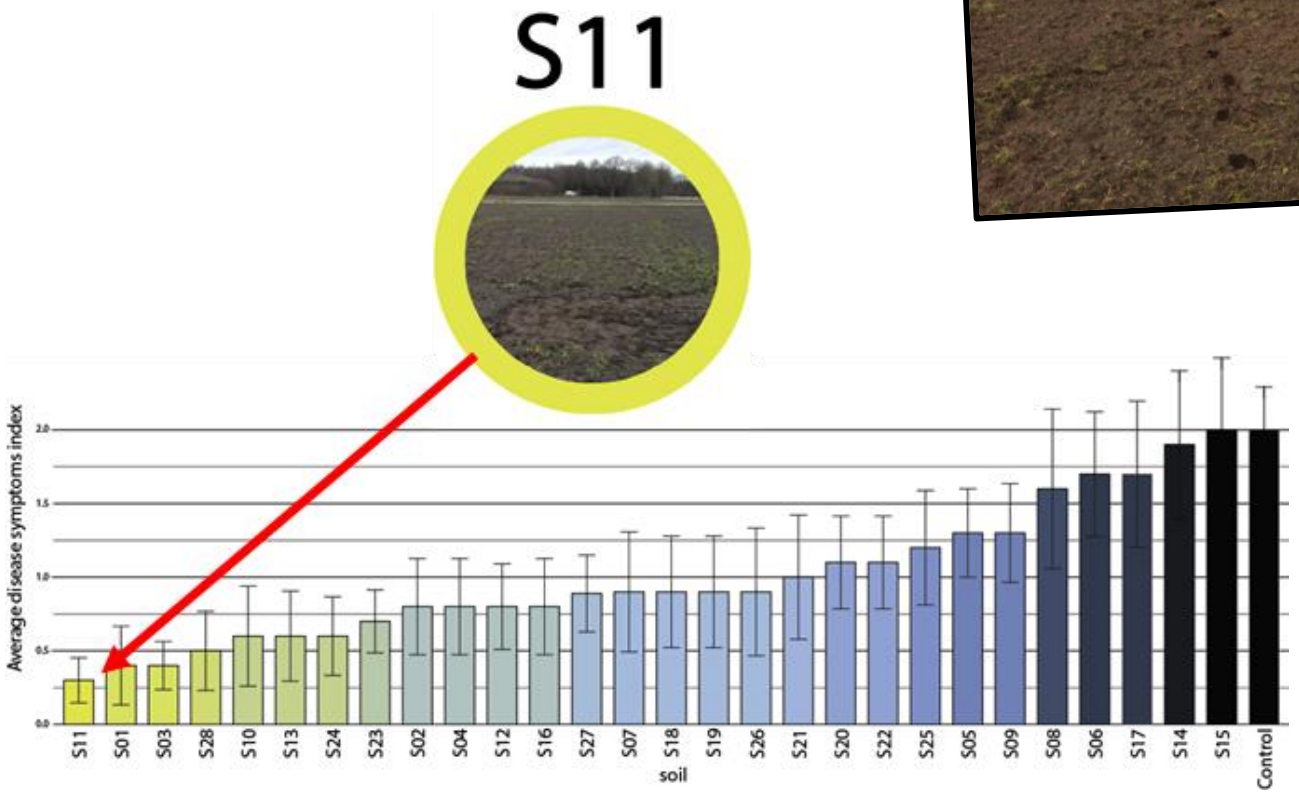
What makes a soil suppressive ?

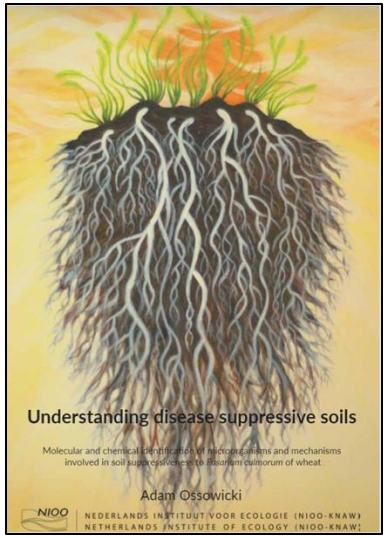
What are the key microbial taxa involved ?

What metabolites are produced ?

What are key microbial functions involved ?

Deciphering the microbiome of disease suppressive soils by dilution-to-extinction





More...

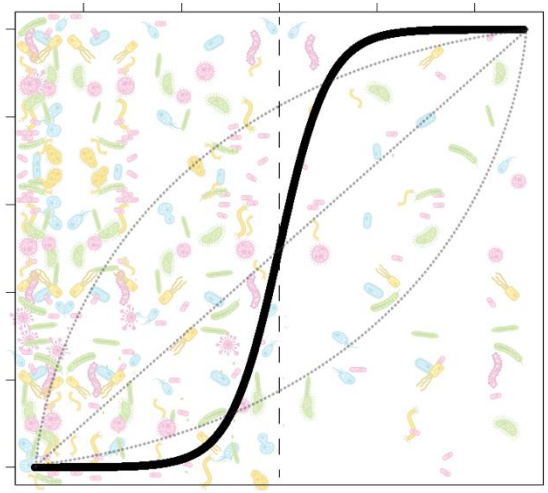
Disentangling soil microbiome functions by perturbation

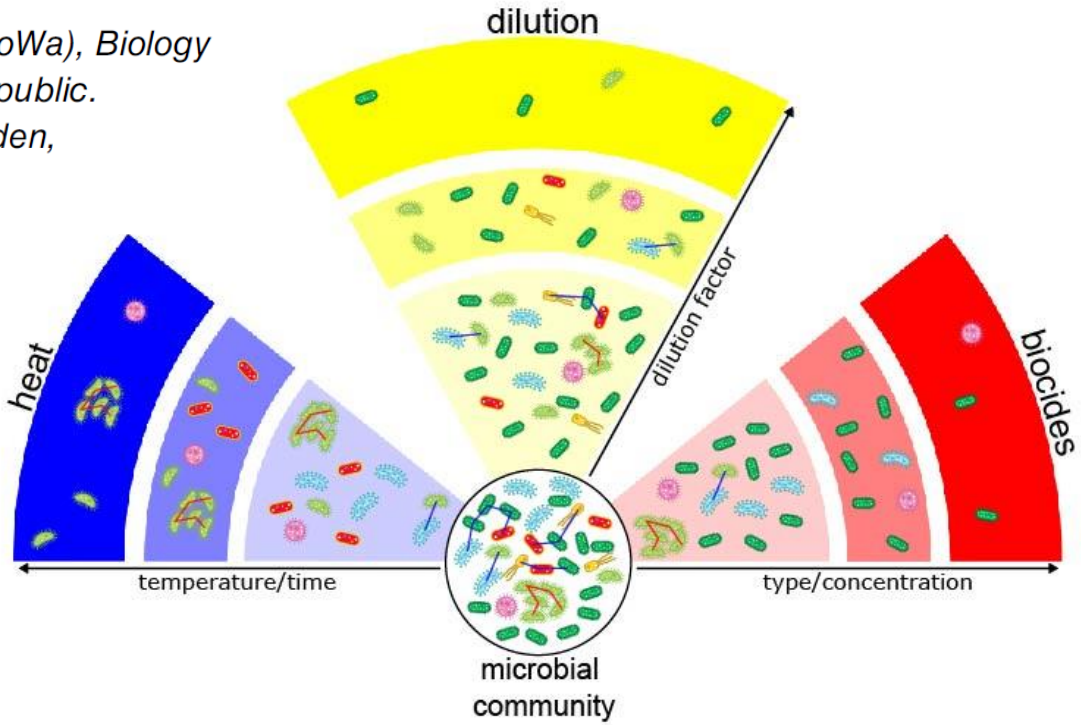
Adam Ossowicki, [1,2*] **Jos M. Raaijmakers** [1,3] **and Paolina Garbeva** [1]

[1]*Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, Wageningen, PB, 6708, Netherlands.*

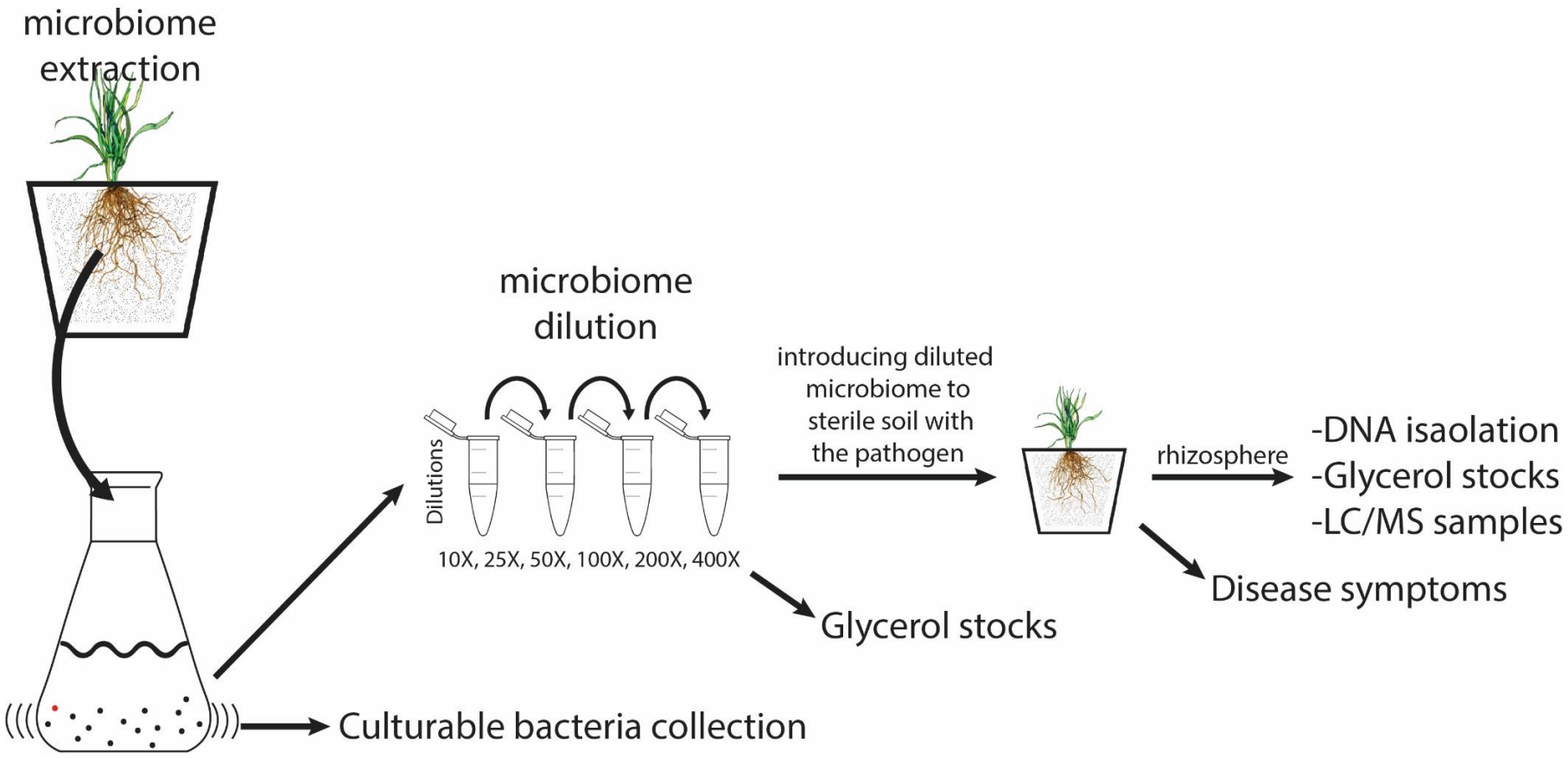
[2]*Soil and Water Research Infrastructure (SoWa), Biology Centre CAS, České Budějovice, Czech Republic.*

[3]*Institute of Biology, Leiden University, Leiden, Netherlands.*

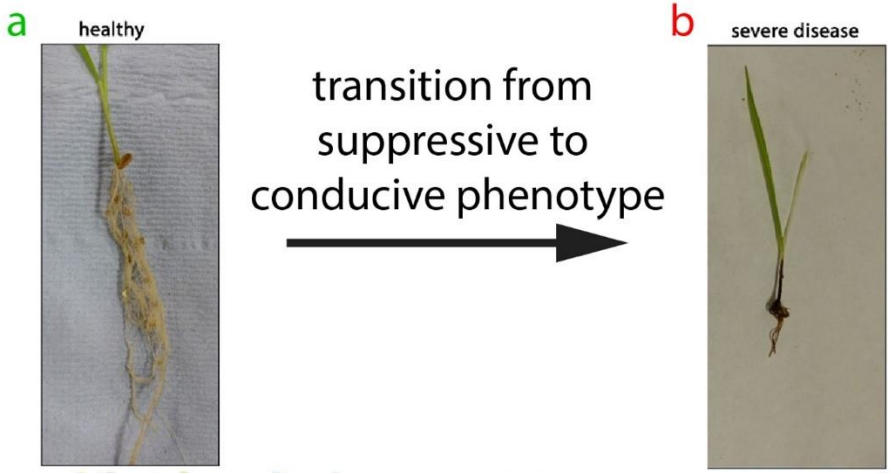


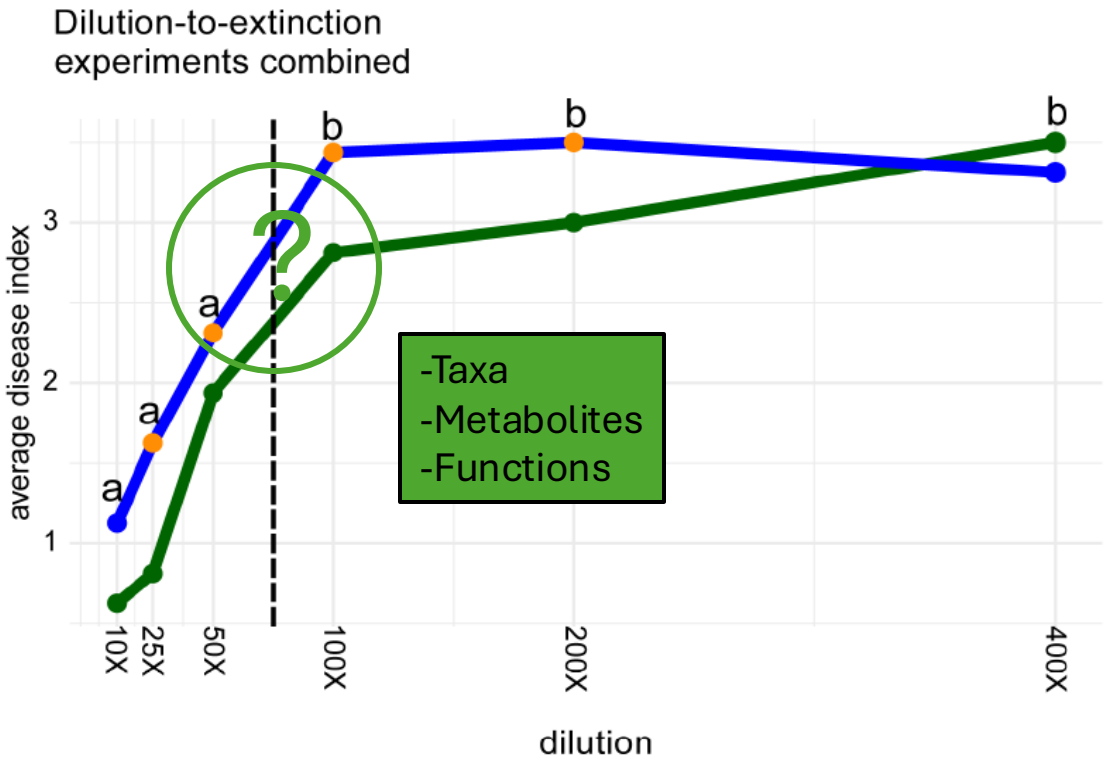


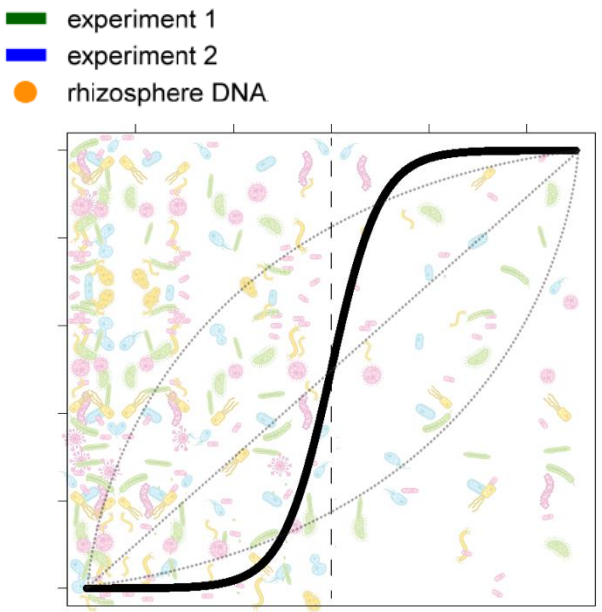
dilution-to-extinction - setup



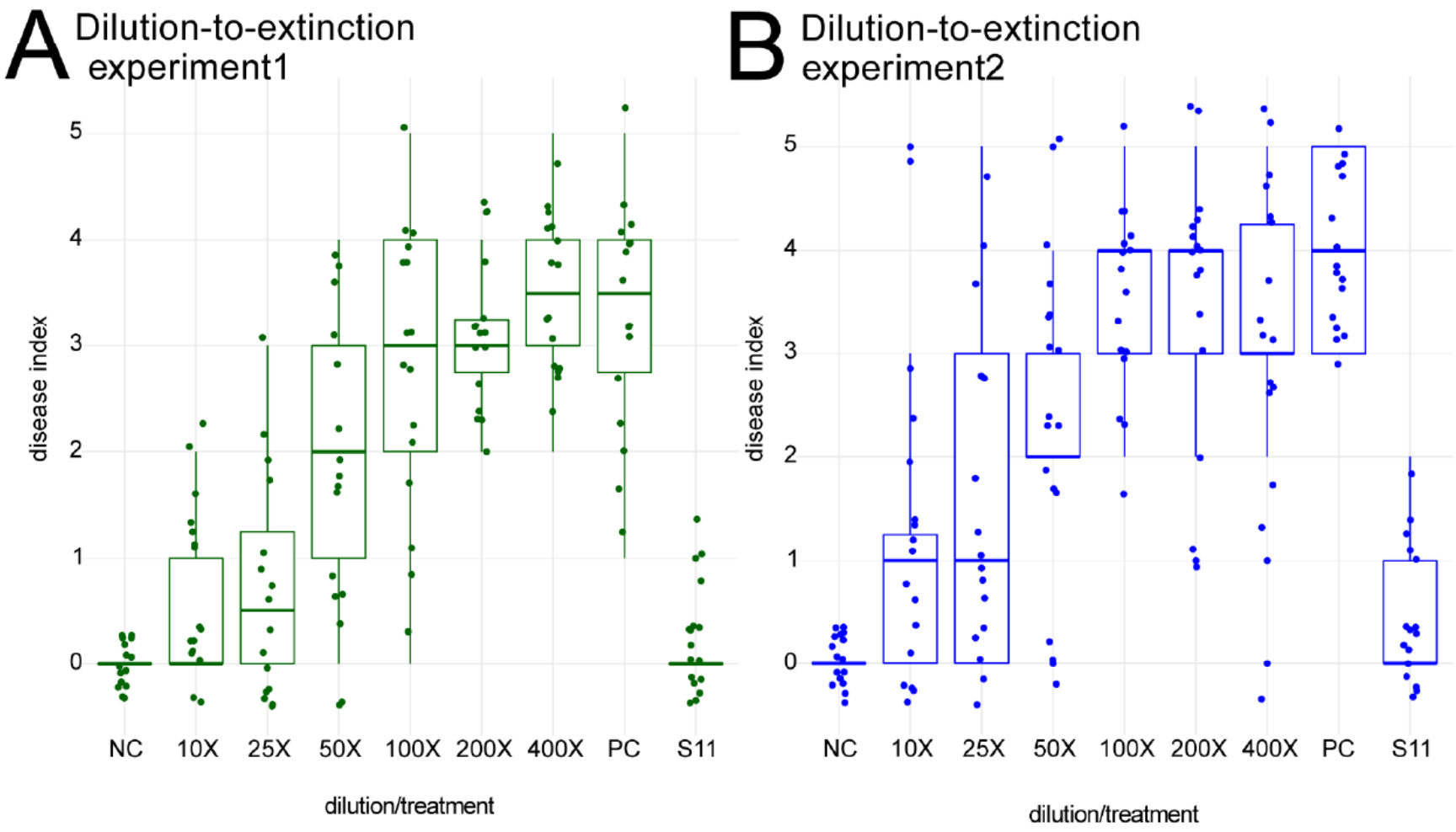
dilution-to-extinction - Results





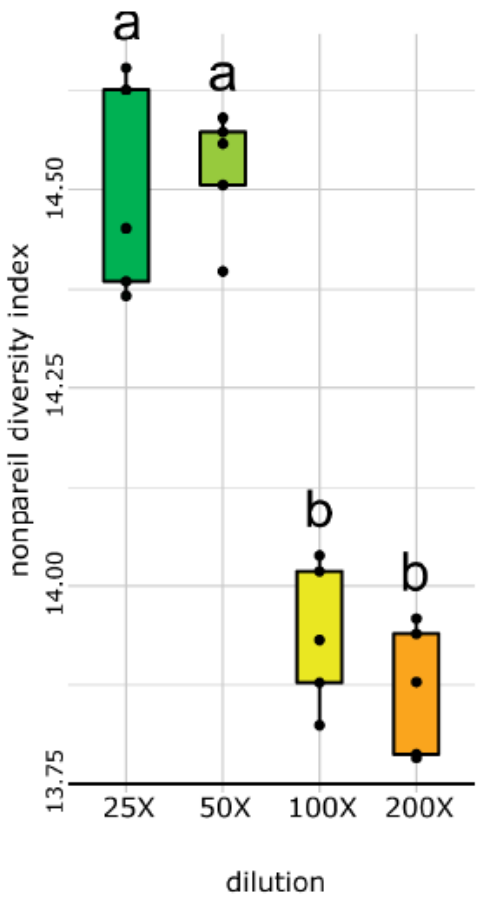


dilution-to-extinction - Results

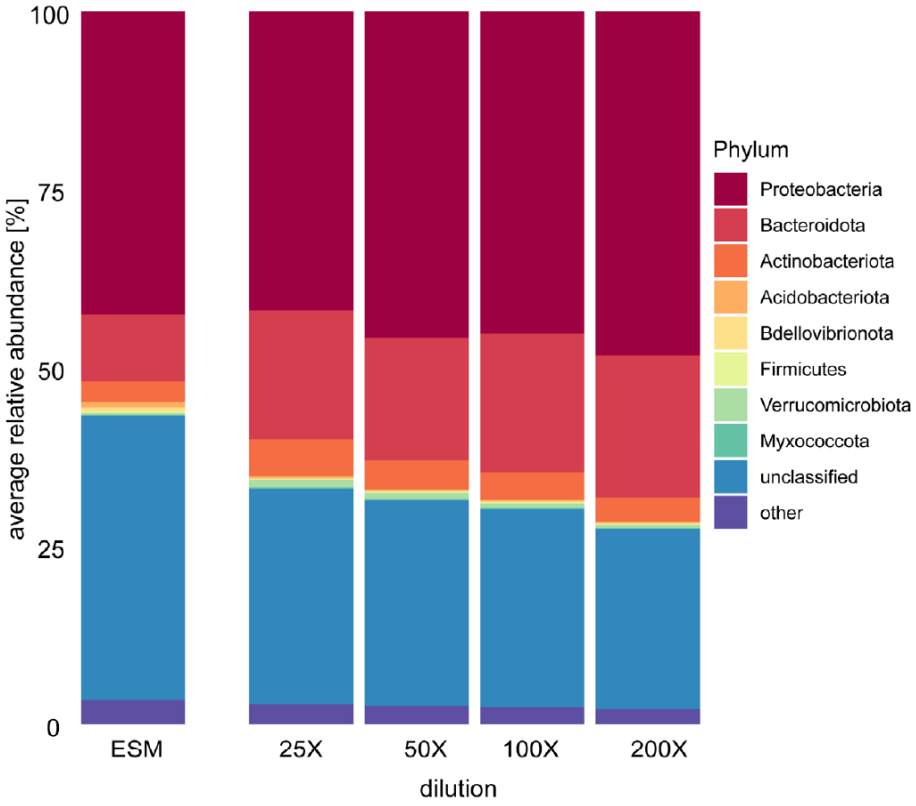


dilution-to-extinction - Results

Diversity



Taxonomic distribution

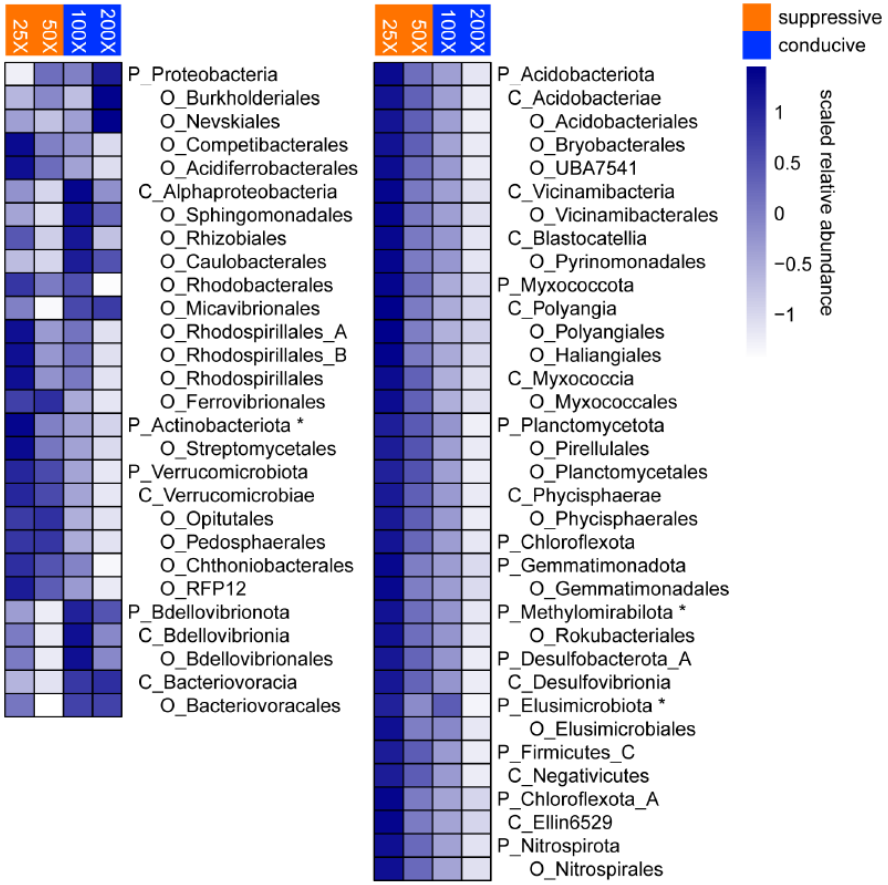


Nonpareil index: Rodriguez-R, L. M. et al. mSystems 2018

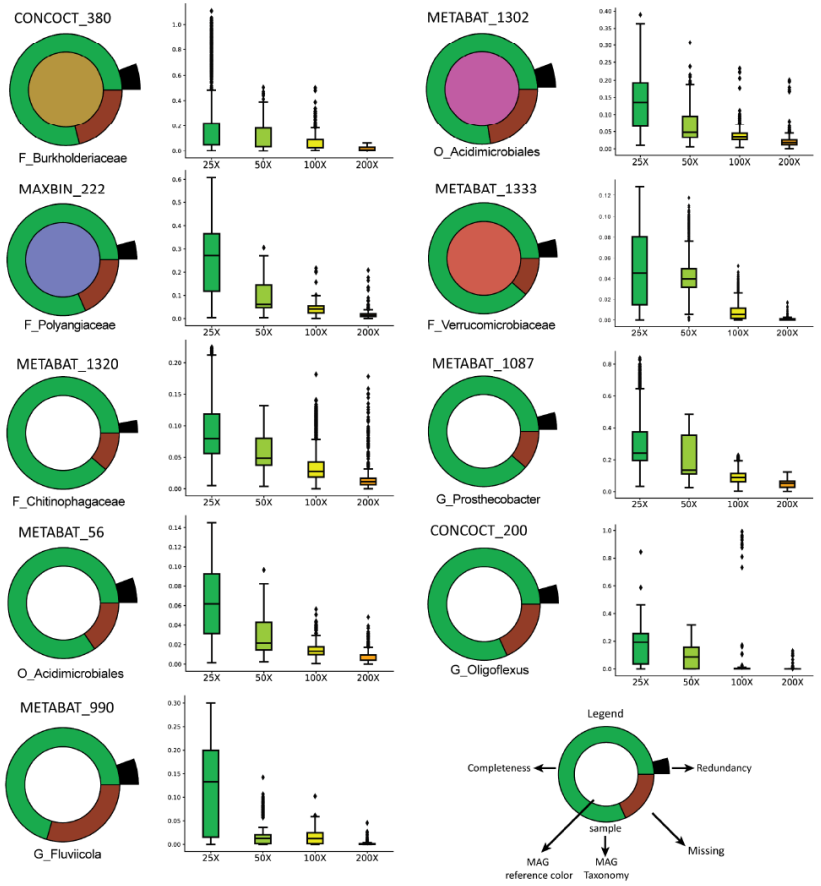
dilution-to-extinction - Results

Taxonomic distribution

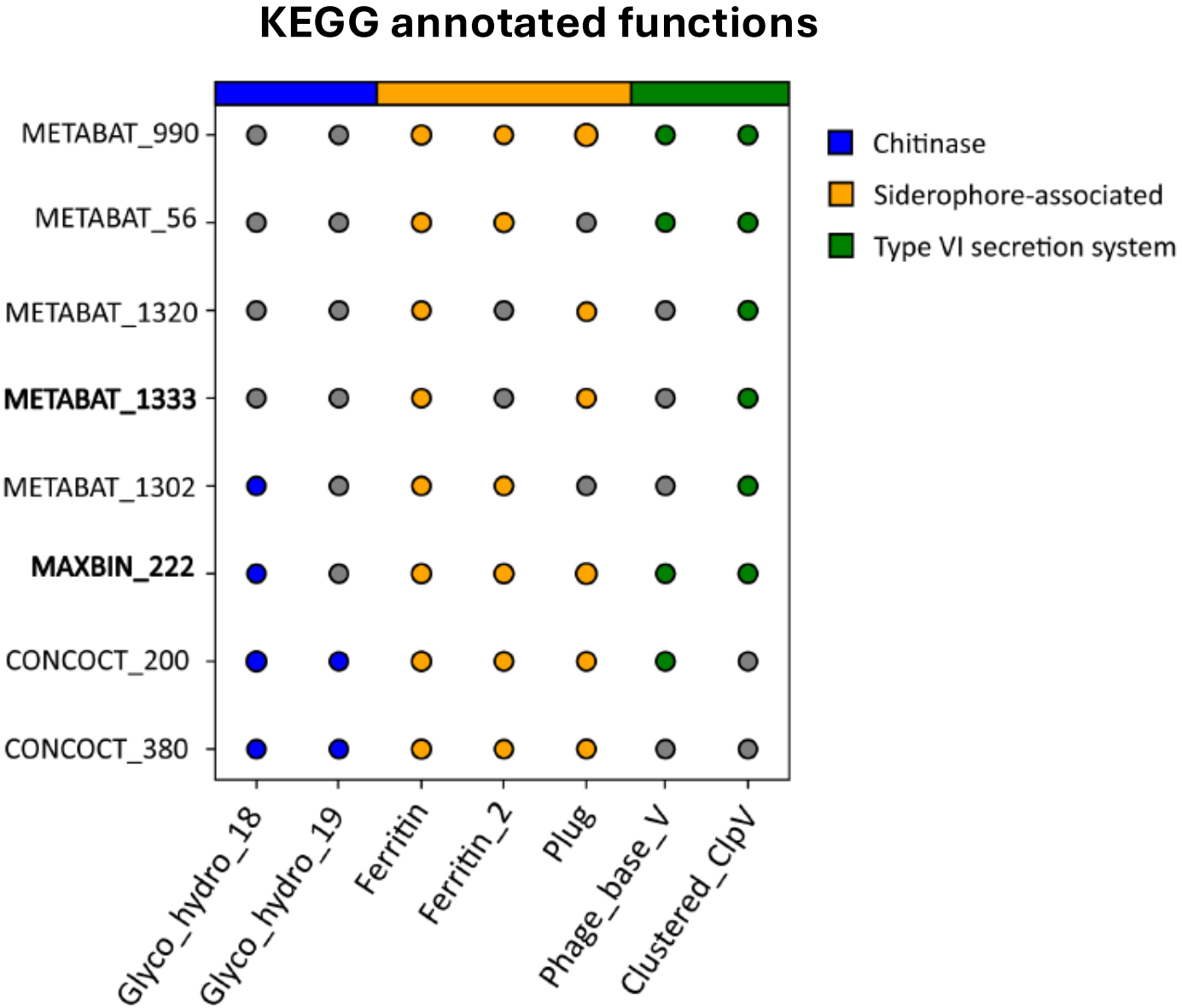
– lower levels



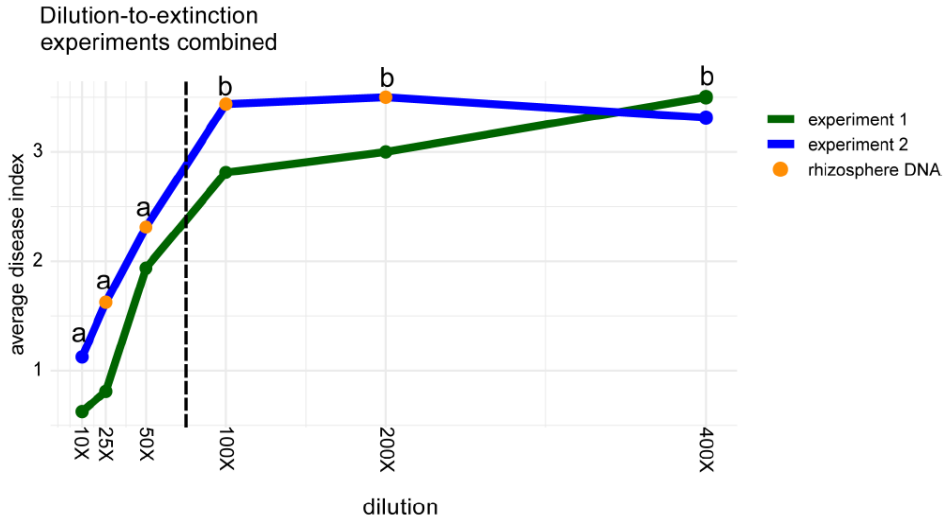
Binning



dilution-to-extinction - Results



dilution-to-extinction - summary



Brief summary:

- Both diversity and abundance of specific bacteria groups plays a role in the *F.culmorum* suppressive soil
- Functional analysis of the rhizosphere metagenomes revealed an enrichment of KEGG orthologs associated with iron uptake, chitinases and type 6 secretion systems
- Nine metagenome-assembled genomes were associated with the diminished suppressive phenotype

Validation study !

What now ?

Linking phenotypic and metagenomic data with …

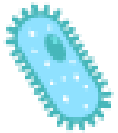
- ~350 sequenced bacteria isolates

- Myxobacteria isolates

  (Rolf Müller's group HIPS, Saarbrücken, Germany)

- Metabolomic data

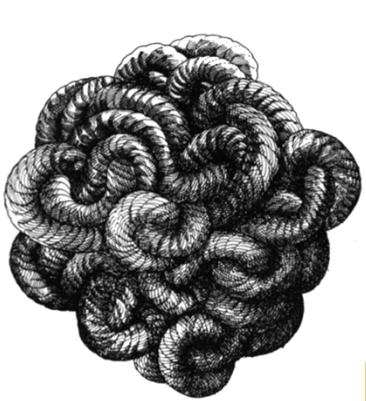
- Designing synthetic communities and testing them

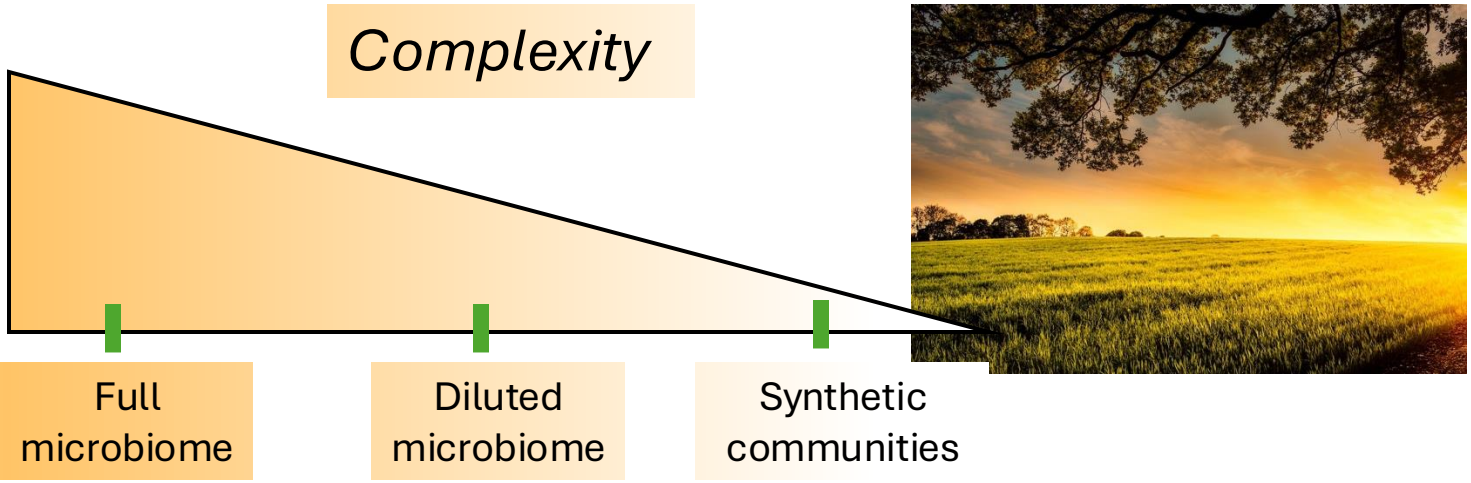


- Transcriptomics on synthetic communities to verify BGC expression and CRISPR-Cas validation

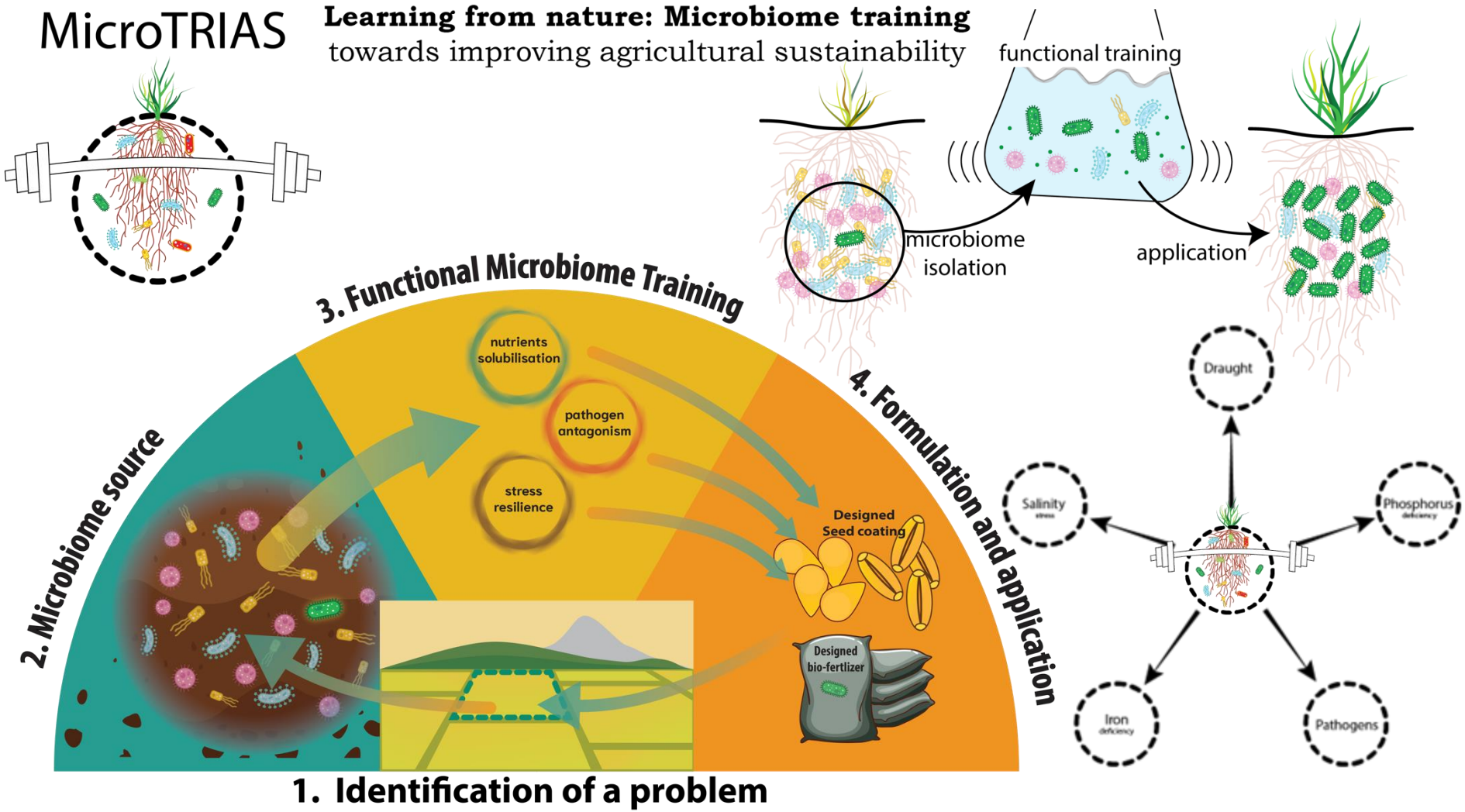
Working title:
Combinatorial design of synthetic communities will allow to identify chemical interaction networks in the microbiome

Ossowicki, Tracanna, Jing et al. *in preparation*



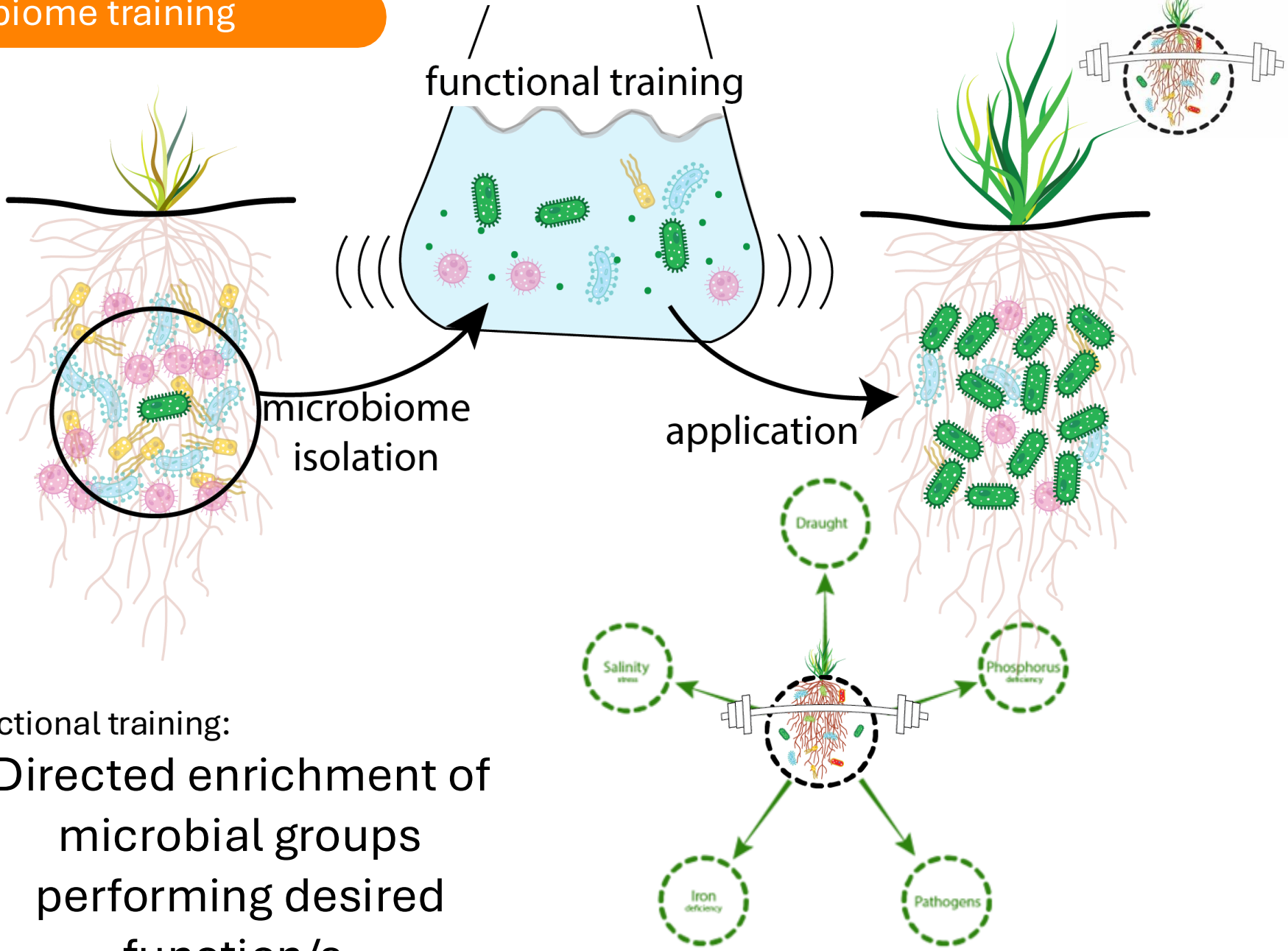


Microbiome training



Microbiome training

MicroTRIAS



Functional training:

Directed enrichment of microbial groups performing desired function/s.

Thank you for your attention

Paolina Garbeva
Jos Raaijmakers
Jiayi Jing

Marnix Medema
Vittorio Tracanna
Zach Reitz

Gilles van Wezel
Somaya Elsayed

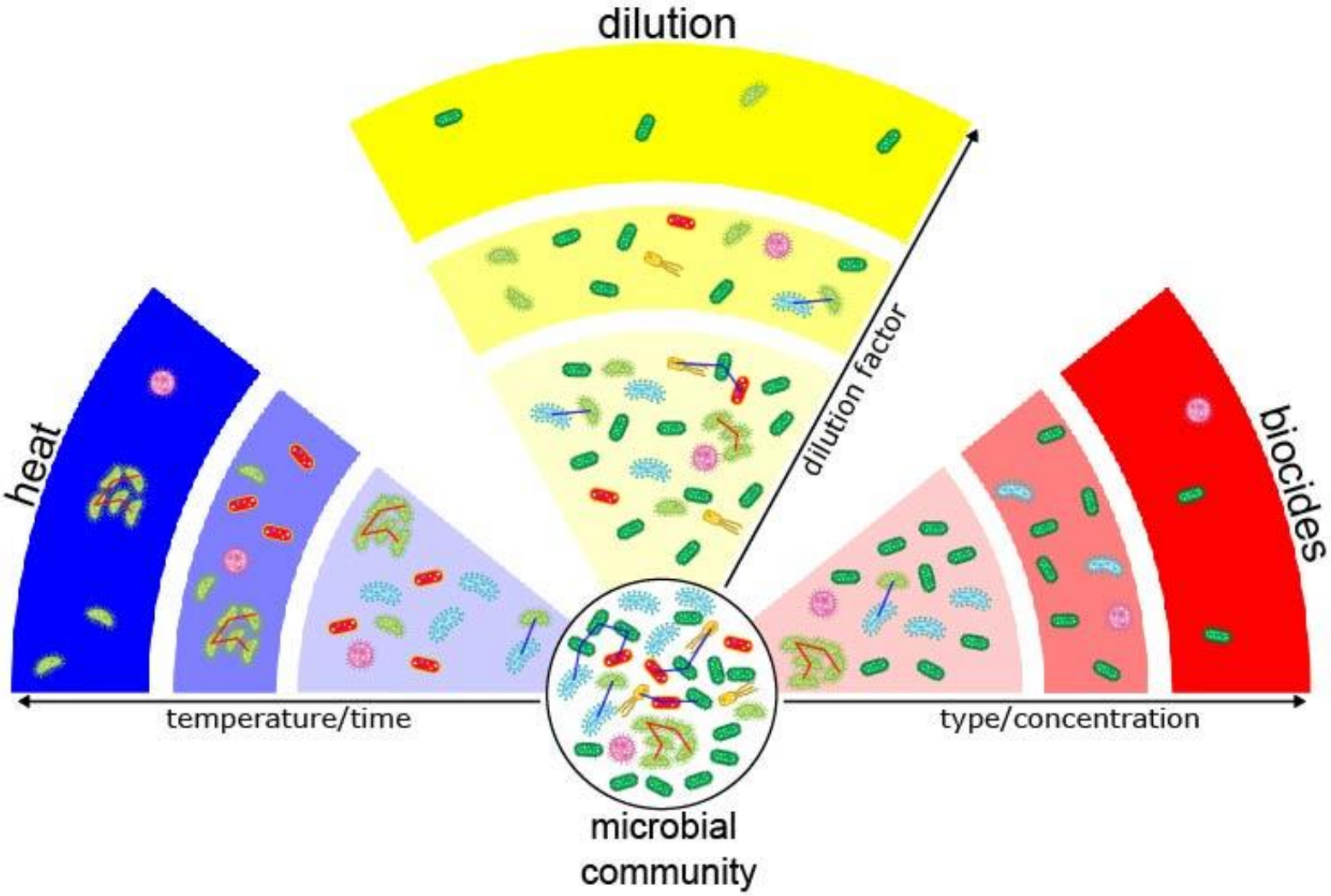
Pilar Puente
Bruno Pollet

Victor Carrion
Pascal Nuijten
Kevin Bretscher

Roey Angel
Alica Chronakova

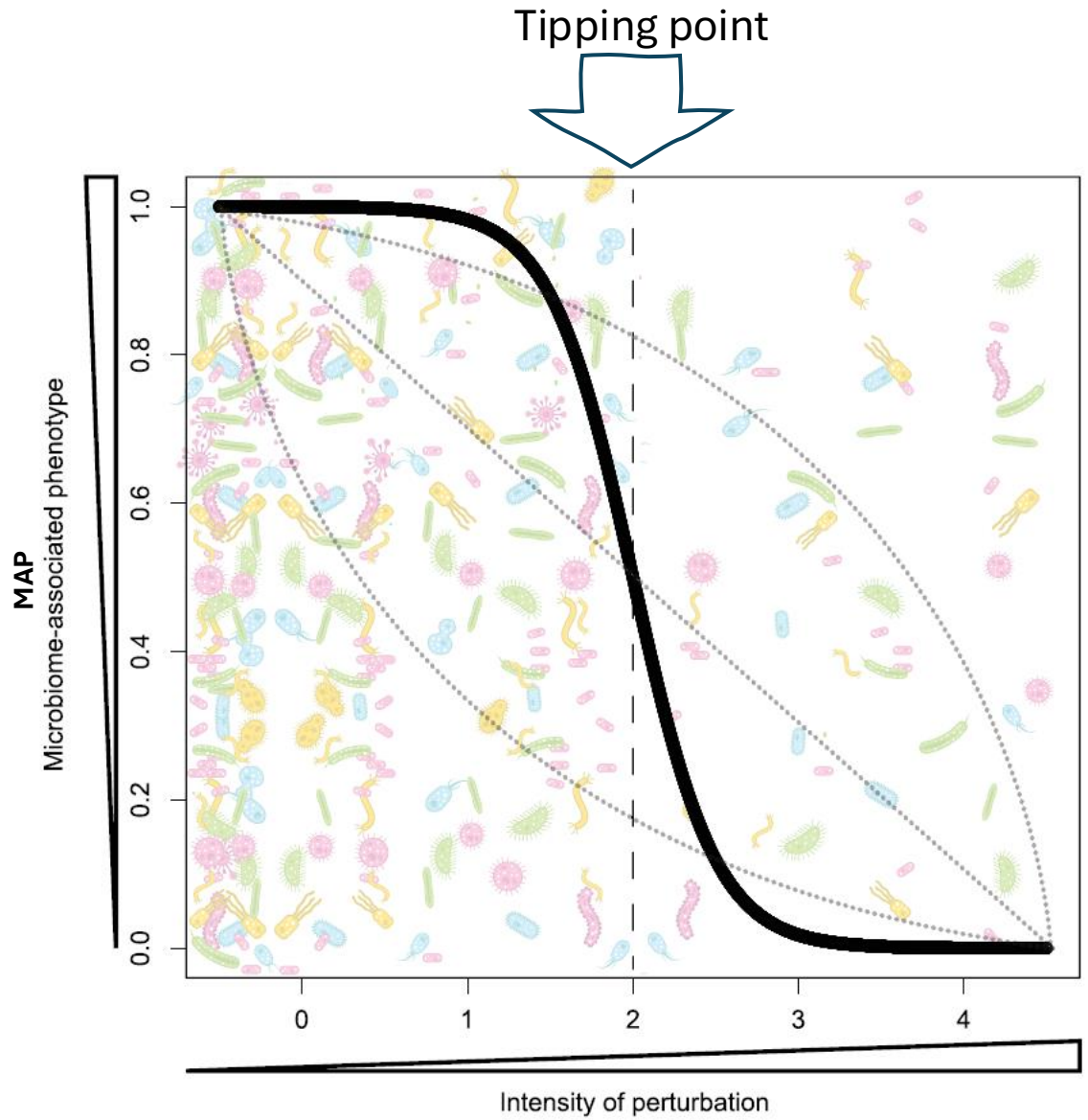
Marie Skłodowska-Curie Actions
Postdoctoral Fellowship

Perturbation – how to simplify a microbiome



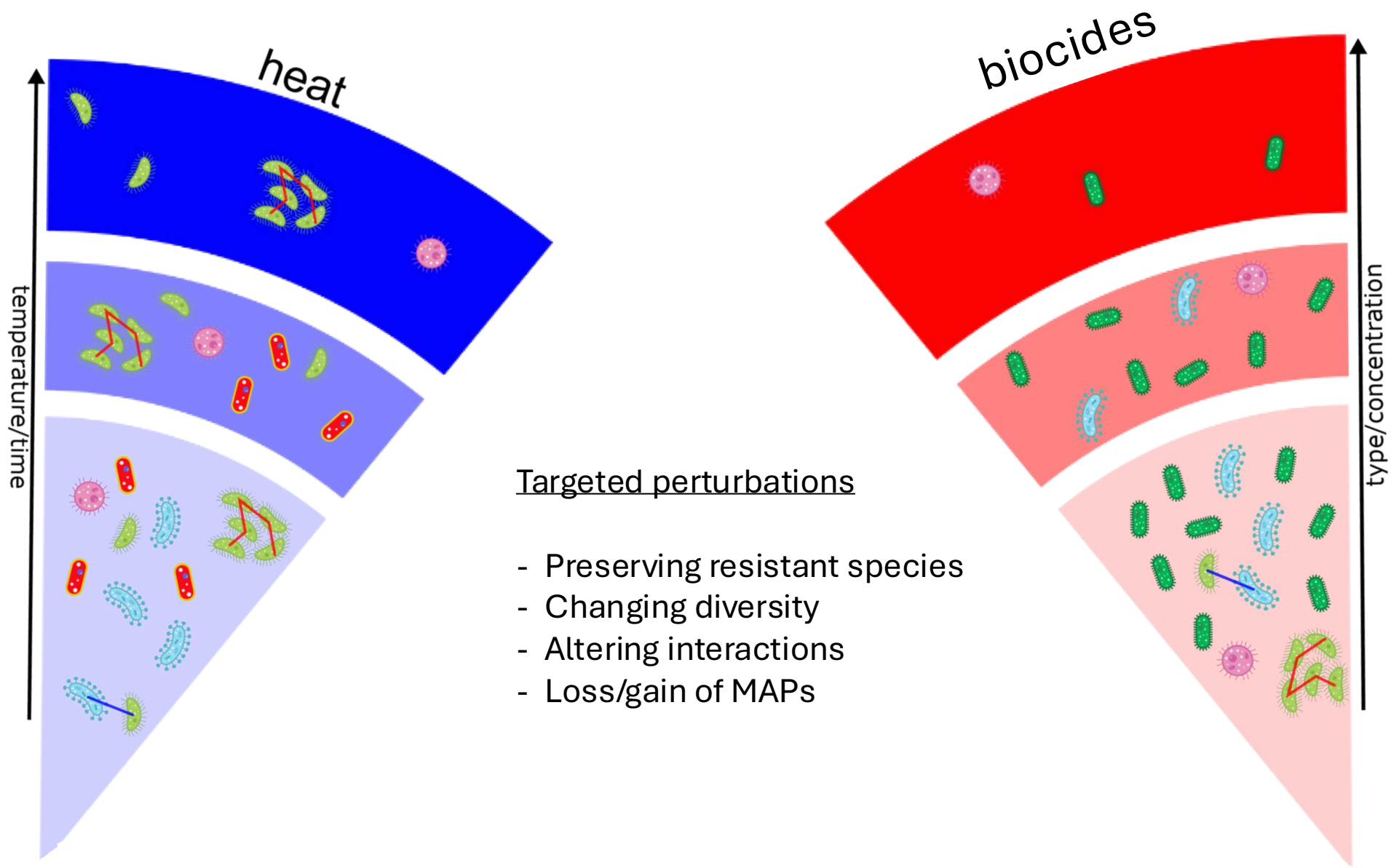
Ossowicki at al. Environ. Microbiol. Rep. 2021

Perturbation analysis - phenotypes



Ossowicki at al. Environ. Microbiol. Rep. 2021

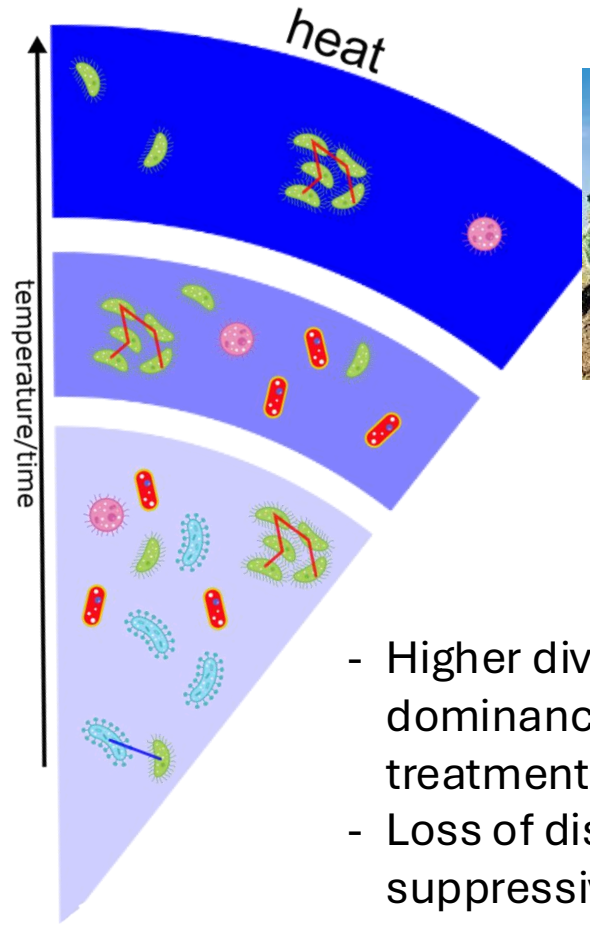
Perturbation analysis of soil microbiomes

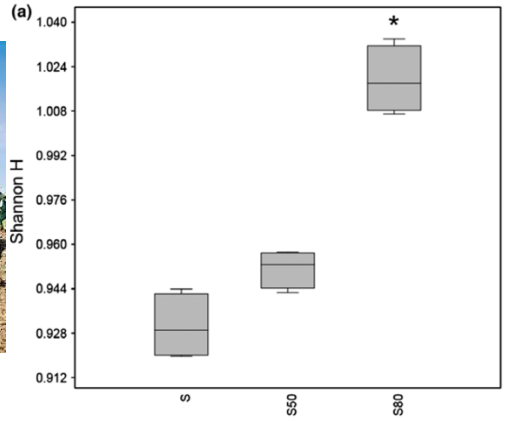


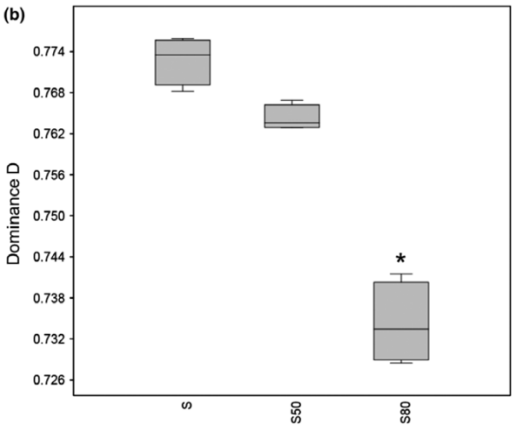
Targeted perturbations

- Preserving resistant species
- Changing diversity
- Altering interactions
- Loss/gain of MAPs

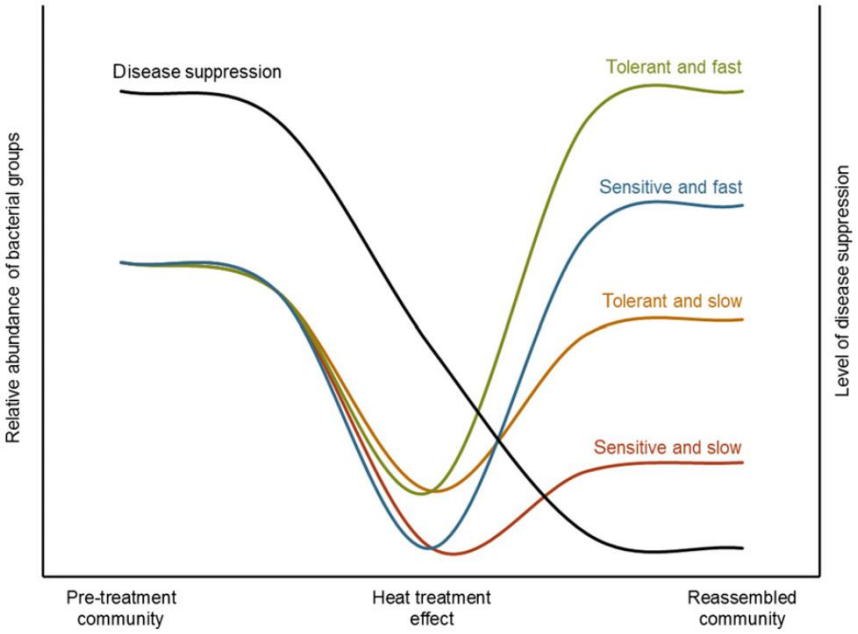
Perturbation analysis of soil microbiomes





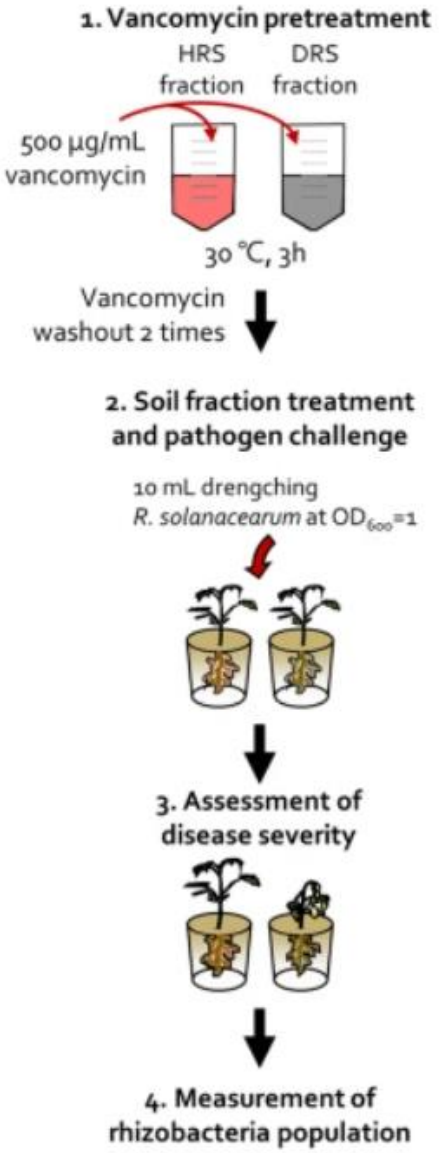


- Higher diversity and lower dominance after hear treatment
- Loss of disease suppressiveness
- Identified the potential role of *Acidobacteria* and *Actinobacteria*

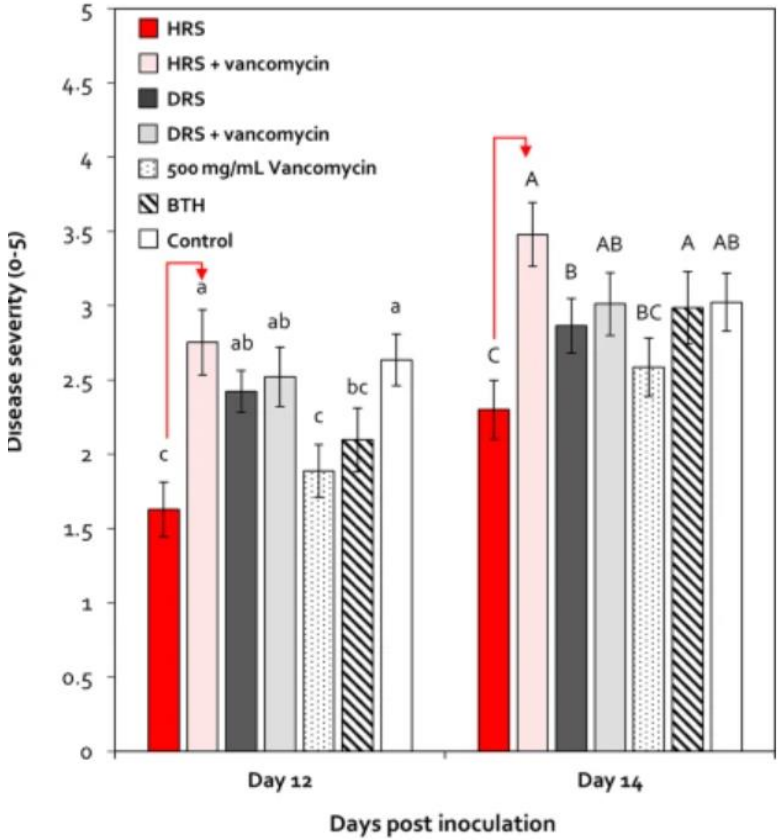


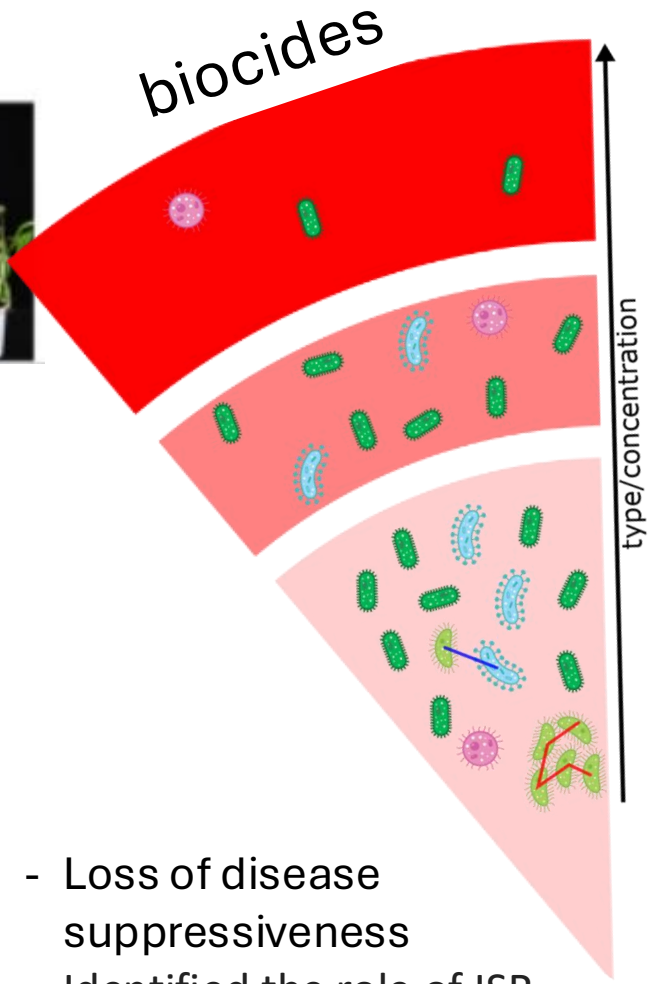
van der Voort et al. Ecology Letters

Perturbation analysis of soil microbiomes



bacterial wilt in tomato

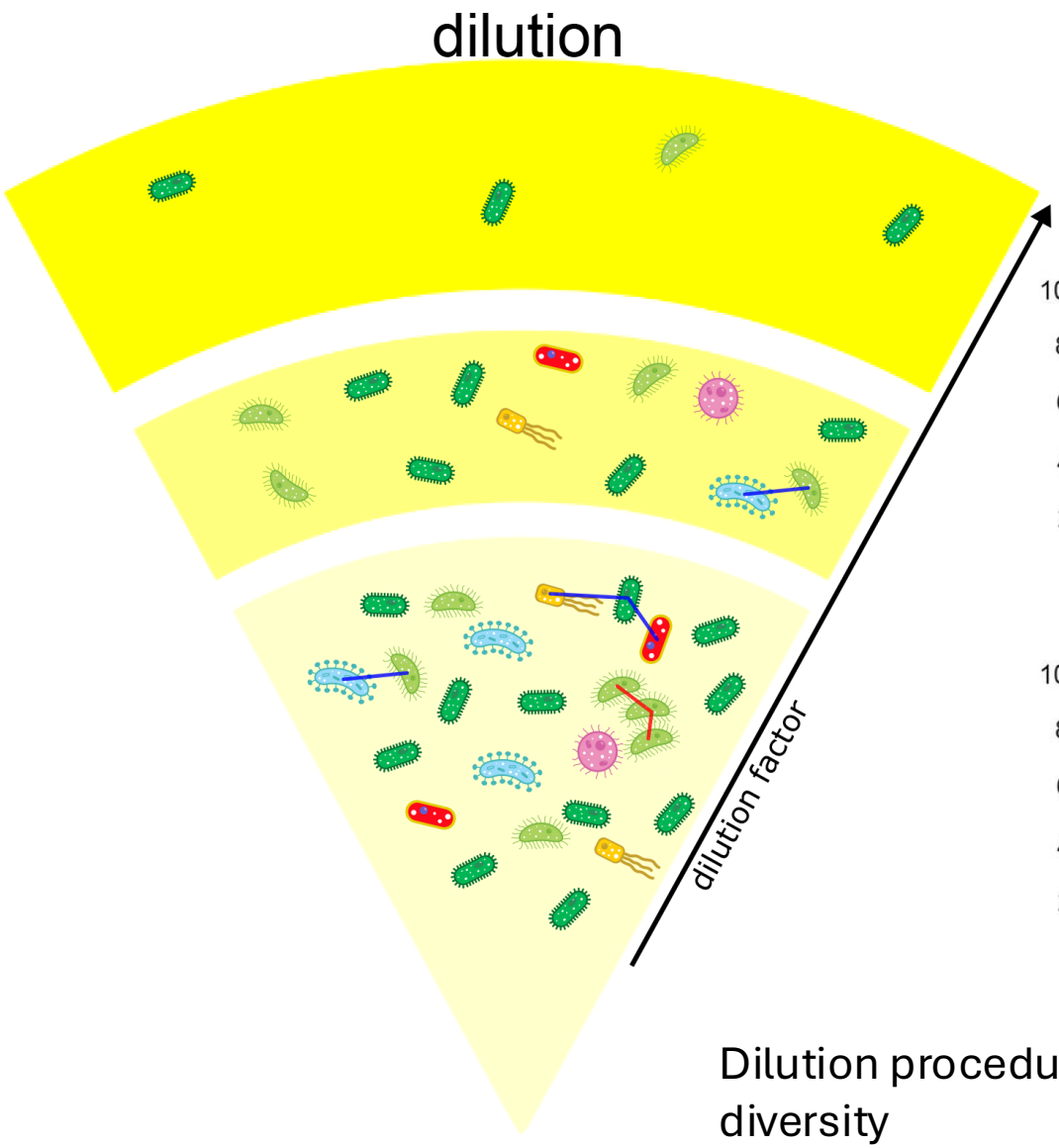




- Loss of disease suppressiveness
- Identified the role of ISR-eliciting Firmicutes and Actinobacteria

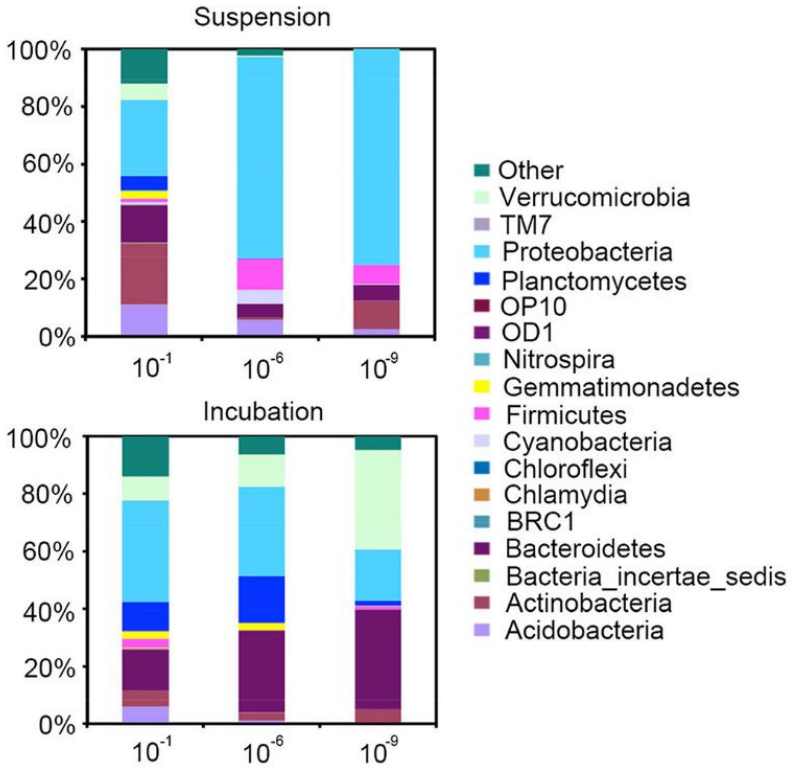
Lee at al. ISME 2020

Perturbation analysis of soil microbiomes



Untargeted perturbation

- With extraction and without extraction
- Loss/gain of MAPs
- Decreasing diversity

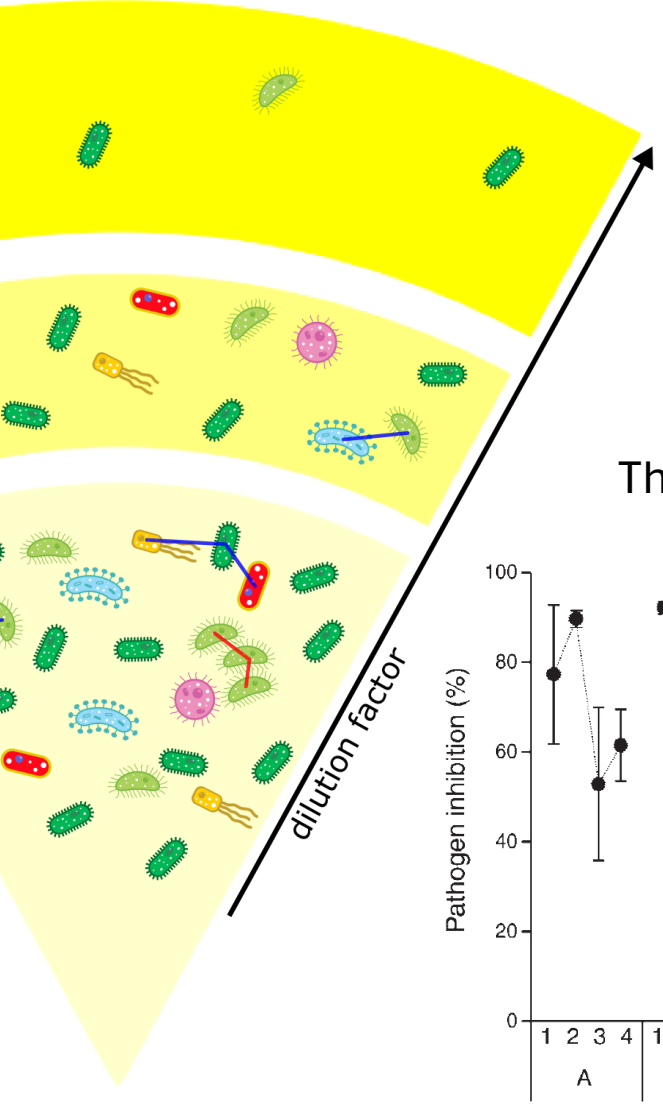


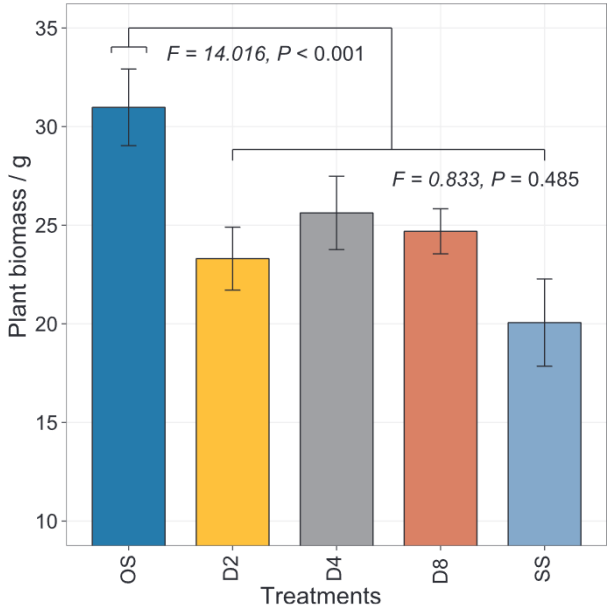
Dilution procedure leads to a reduction of bacterial diversity
and a change in relative abundance

Yan at al. AEM 2015

Perturbation analysis of soil microbiomes

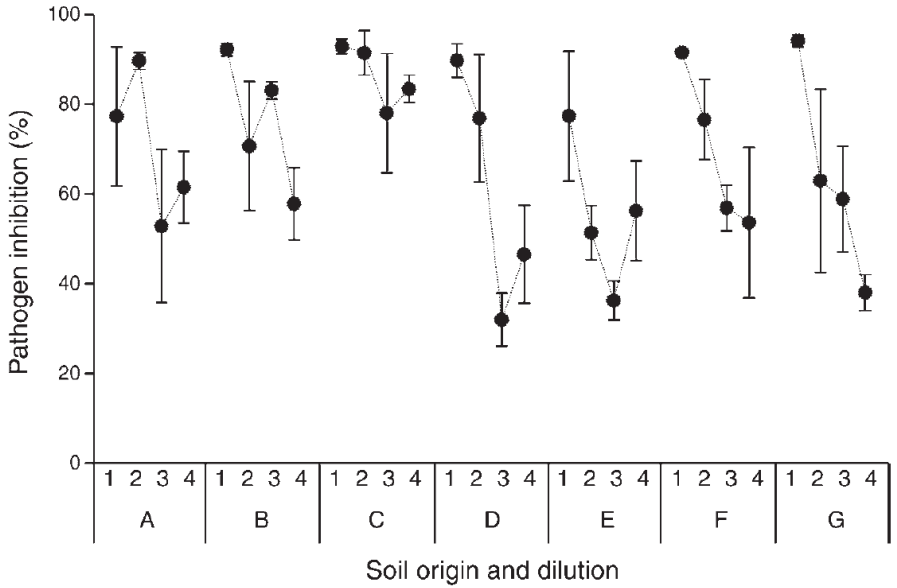
dilution





The dilution of microbiome has a negative effect on plant bioma

Chen et al. Env.Inter. 2020



The dilution of microbiome has a negative effect volatile related inhibition of pathogens

Hol et al. Ecology 2015

More...

Disentangling soil microbiome functions by perturbation

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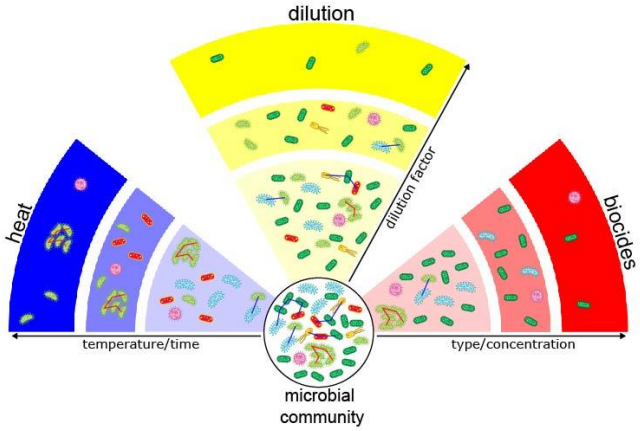
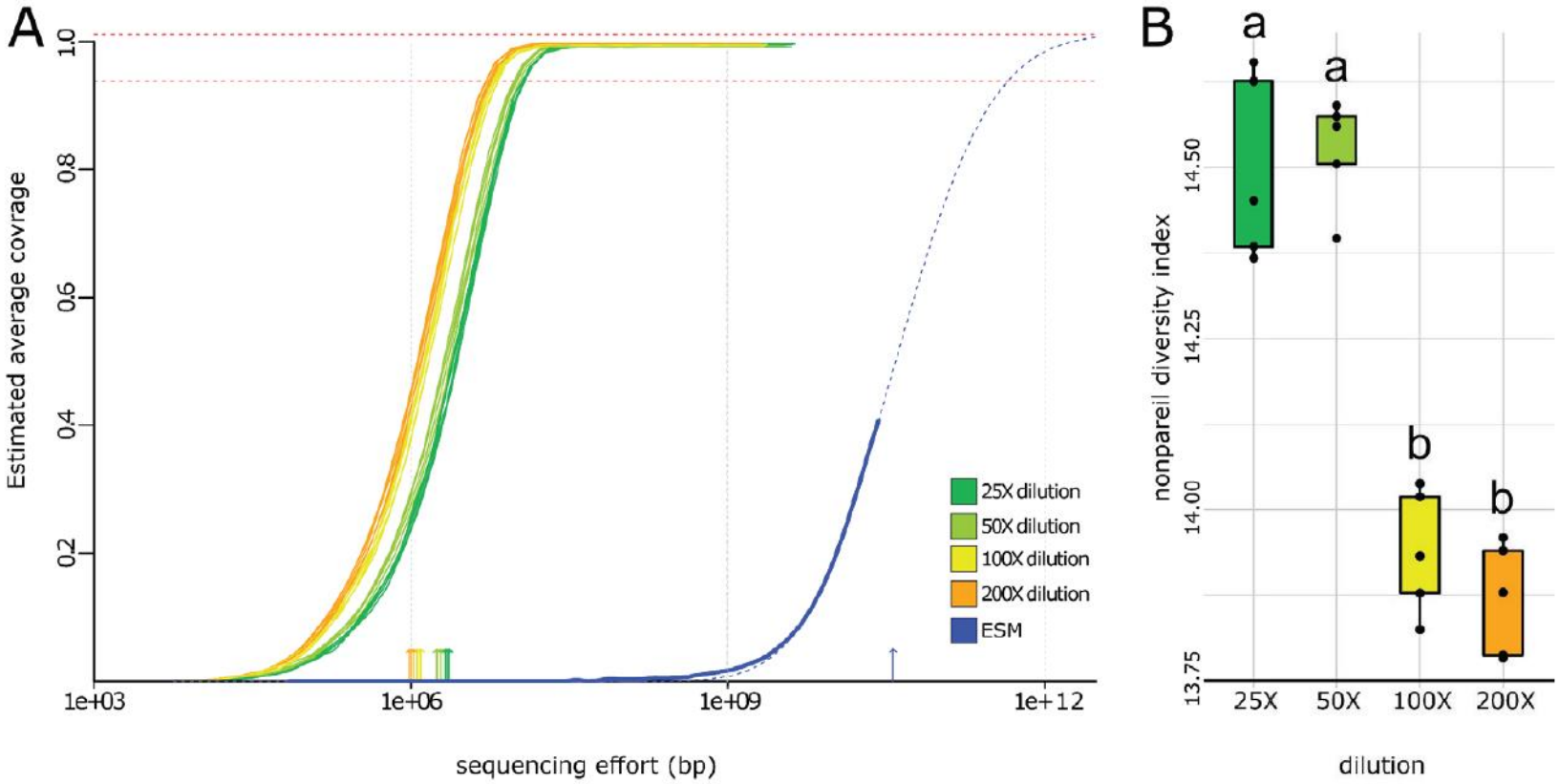
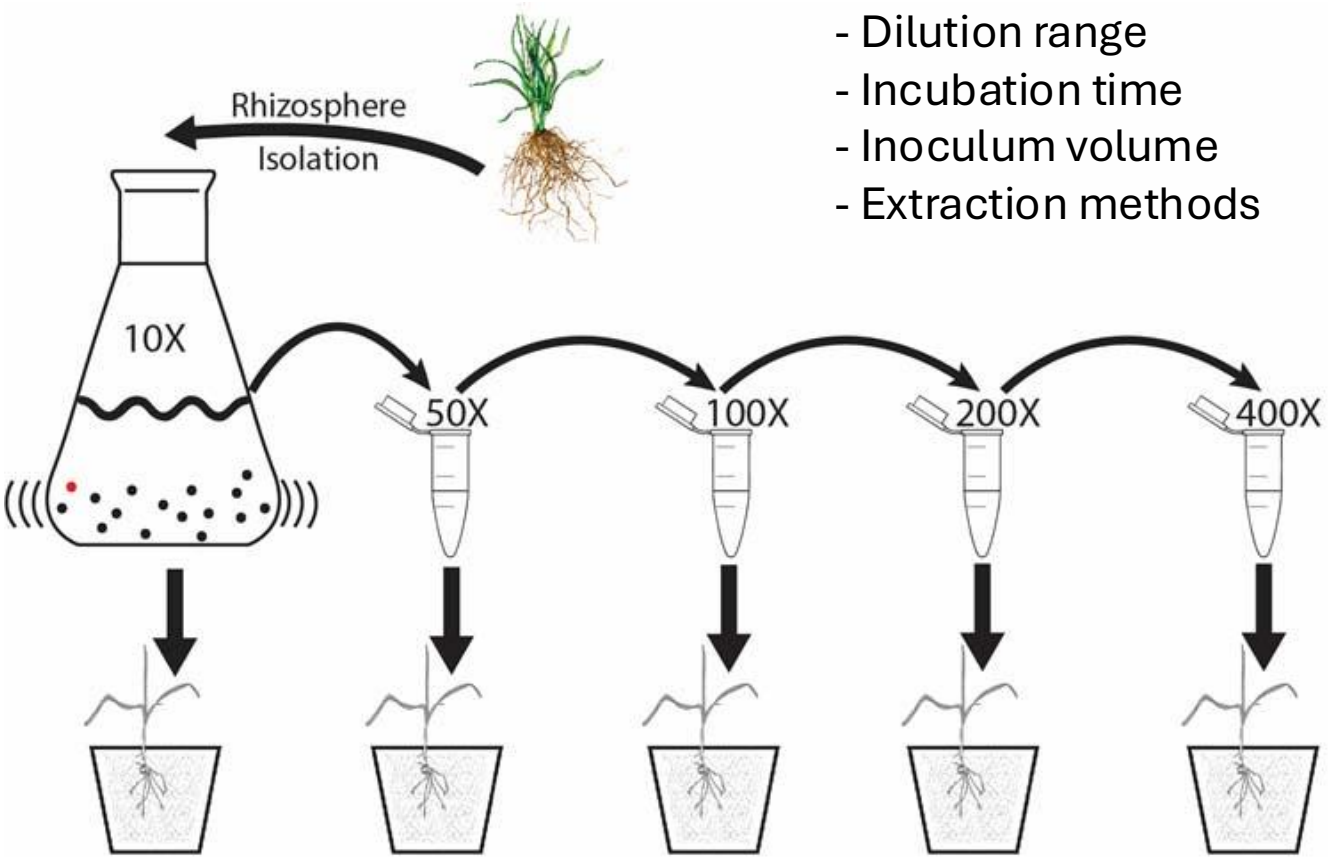



A
Estimated average covrage
1.0
0.8
0.6
0.4
0.2
1e+03
1e+06
1e+09
1e+12
sequencing effort (bp)
25X dilution
50X dilution
100X dilution
200X dilution
ESM
B
nonpareil diversity index
14.50
14.25
14.00
13.75
a
a
b
b
25X
50X
100X
200X
dilution

Deciphering the microbiome of disease suppressiv soils by dilution-to-extinction

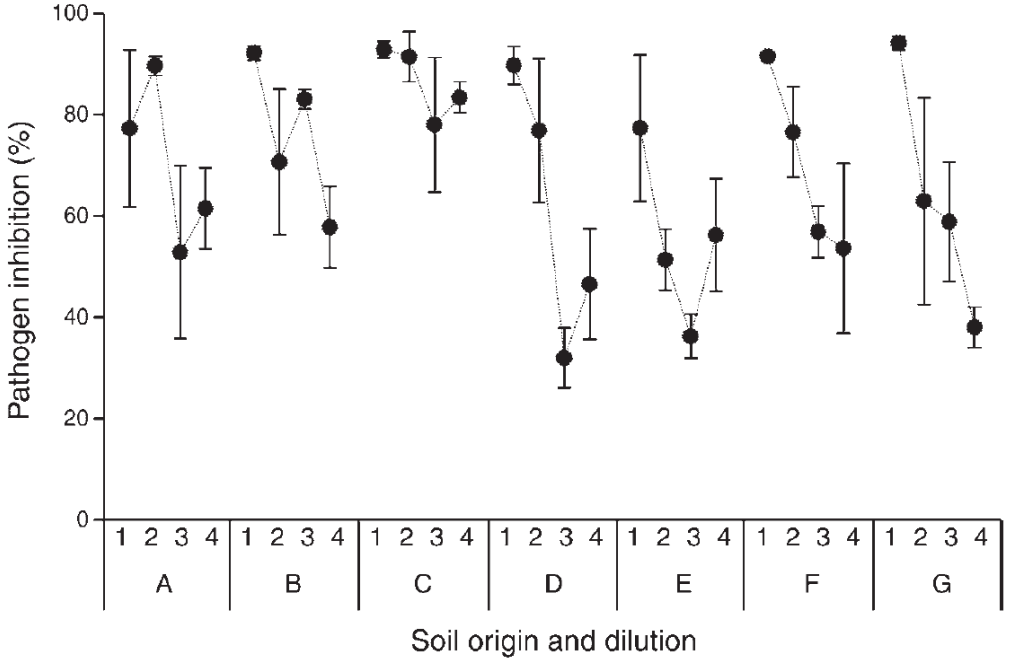


Optimization:
- Dilution range
- Incubation time
- Inoculum volume
- Extraction methods

Perturbation analysis

Non-random species loss in bacterial communities reduces antifungal volatile production

W. H. Gera Hol,[1,4] Paolina Garbeva,[2] Cornelis Hordijk,[2] Maria P. J. Hundscheid,[2] Paulien J. A. Klein Gunnewiek,[2] Maaike van Agtmaal,[2] Eiko E. Kuramae,[2] and Wietse de Boer[2,3]



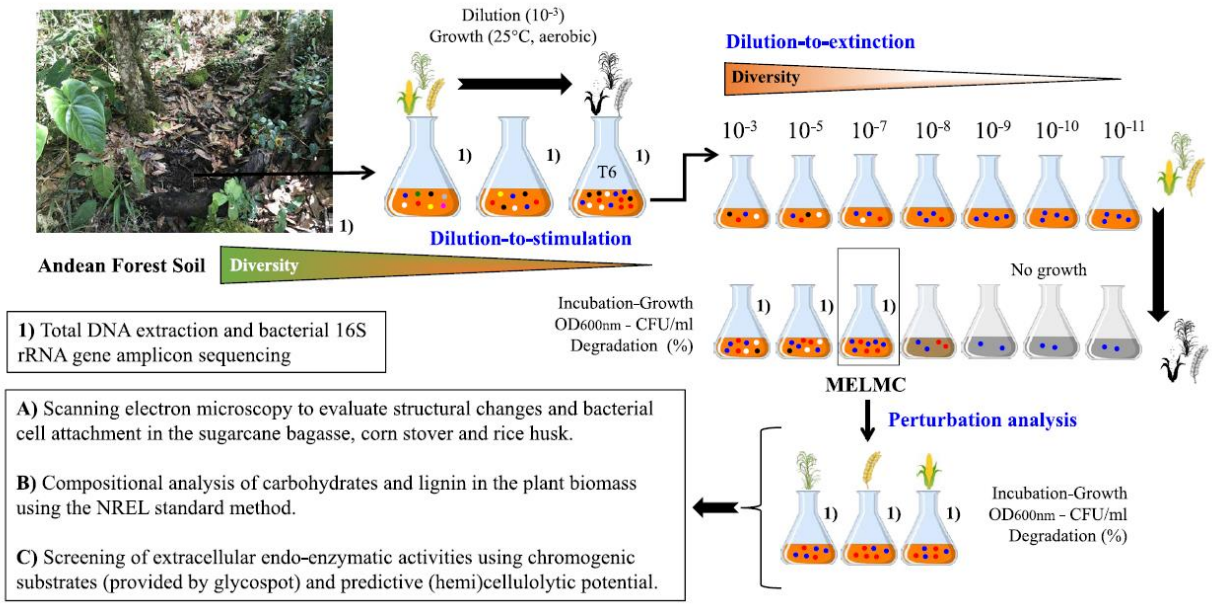
-Reduced pathogen inhibition
-Reduced diversity

Hol et al. Ecology 2015

Perturbation analysis

Dilution-to-Stimulation/Extinction Method: a Combination Enrichment Strategy To Develop a Minimal and Versatile Lignocellulolytic Bacterial Consortium

Laura Díaz-García,[a] Sixing Huang,[b] Cathrin Spröer,[b] Rocío Sierra-Ramírez,[c] Boyke Bunk,[b] Jörg Overmann,[b,d] Diego Javier Jiménez[a]

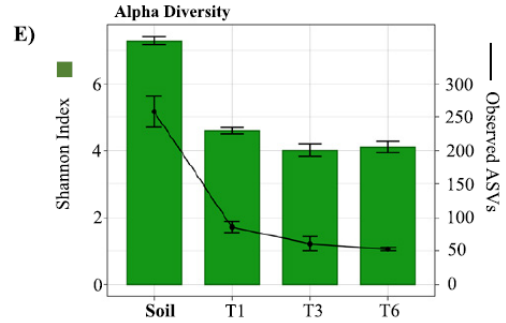


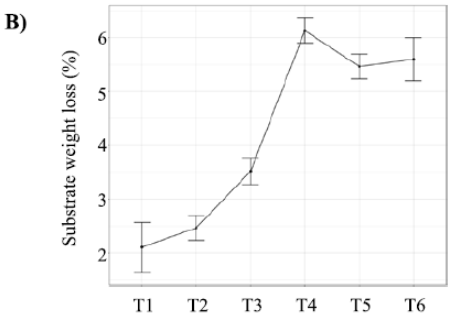
1) Total DNA extraction and bacterial 16S rRNA gene amplicon sequencing

A) Scanning electron microscopy to evaluate structural changes and bacterial cell attachment in the sugarcane bagasse, corn stover and rice husk.

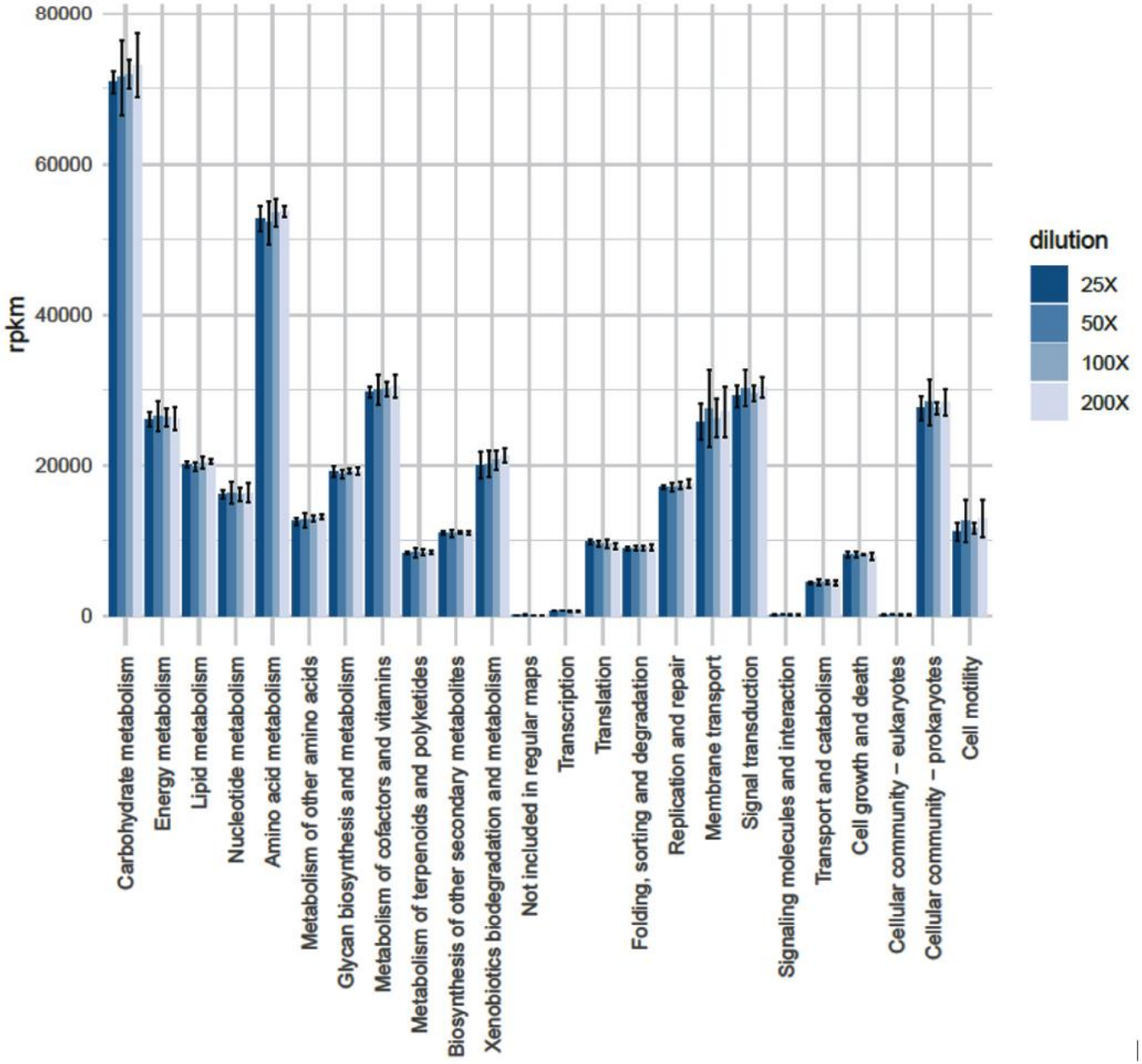
B) Compositional analysis of carbohydrates and lignin in the plant biomass using the NREL standard method.

C) Screening of extracellular endo-enzymatic activities using chromogenic substrates (provided by glycospot) and predictive (hemi)cellulolytic potential.



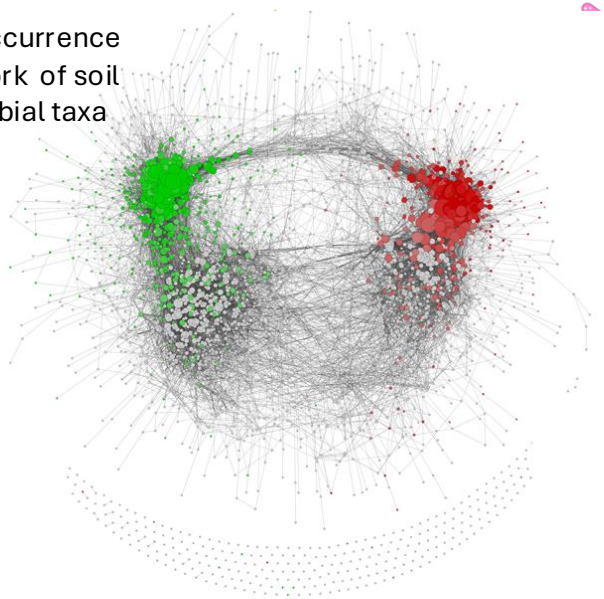


dilution-to-extinction - KEGG

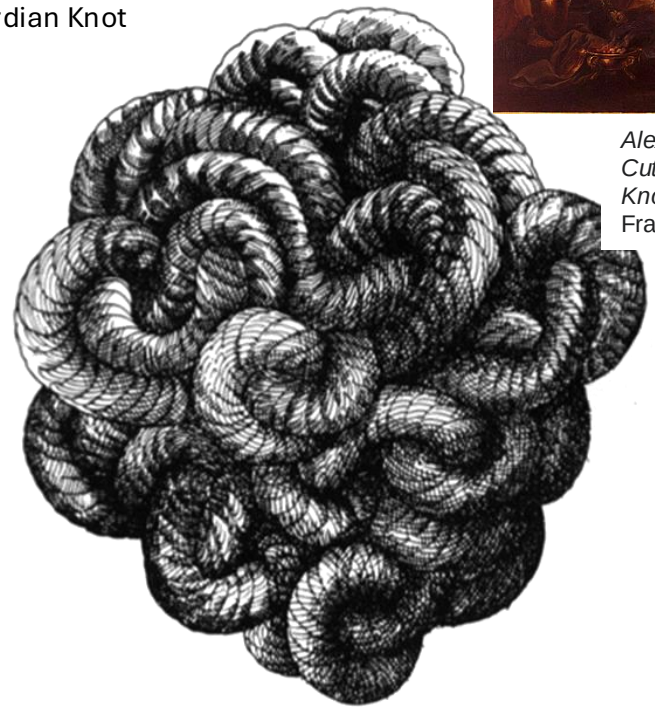


Soil microbiomes – soil suppressiveness

Co-occurrence network of soil microbial taxa



Gordian Knot



Alexander the Great Cutting the Gordian Knot (1767) by Jean-François Godefroy

Is it possible to simplify a microbiome ?