

Using DNA metabarcoding to study plant-microbiote interactions

Ch. Mougel: Genetic basis of plant-microbiote interactions using a GWAS approach

J. Aubert: Bioclustering via latent block model for overdispersed count data: application in microbial ecology



Christophe MOUGEL

Séminaire Math For Genomics
Evry 29 mai 2019

Micro-biote/biome: qu'est ce que c'est ?

- Une communauté microbienne (ou microbiote) est un ensemble d'organismes issus de populations d'espèces distinctes qui cohabitent sur une même aire géographique à un moment donné et qui constituent un réseau d'interactions.
- L'écologie des communautés étudie les patrons de diversité, d'abondance et de composition d'espèces au sein de ces communautés, ainsi que les processus responsables de ces patrons.
- Utilise les processus de génétique des populations : dispersion, sélection, spéciation et dérive.
- Ces processus d'assemblage (notamment spéciation) ont été adaptés aux communautés microbiennes = processus de diversification (intègre diversité phénotypique et fonctionnelle).
- Le microbiote = les individus // microbiome = microbiote dans son environnement.
- Les études actuelles portant sur les interactions plante-microbiote associent des analyses de la diversité taxonomique et fonctionnelle (e.g. From correlations to causalities)

Agroecology

- Societal context
 - Conception of new agricultural practices



and reduced environmental impacts

- F - Grenelle environnement
- F - Ecophyto II 2025
- EU WaterFramework Directive (waiting for those on soil)

The images include: 1. A green banner for 'le Grenelle Environnement' with the text 'Participez au forum'. 2. A small image for 'écophyto2018' showing a plant. 3. An image for 'Ecophyto R&D INRA' featuring a tree and the INRA logo. 4. An image for 'WATER FRAMEWORK DIRECTIVE' showing a fish.

Agroecology

■ Plant are under stresses

Abiotic stresses

Nutrition:
N, P, S



Climate change:
temperature, water



Pollution:
organic and heavy
metals



Cultivated plant

Atlas des Plantes de France,
A.Masclef 1891



Biotic stresses

Competition:
weeds



Bioaggressors:
insects, nematodes
pathogens(viruses,
bacteria, protist,
fungi)

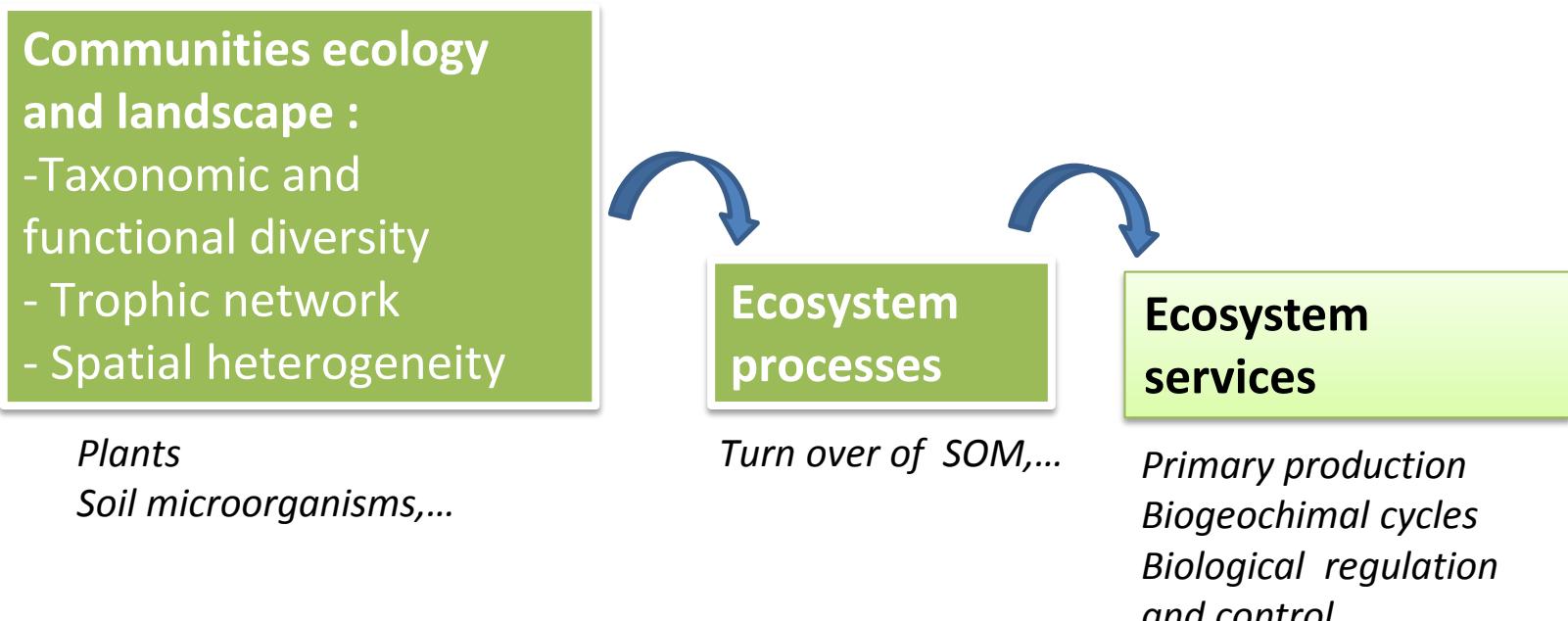


Agronomic context (conventional, organic, biodiversity based farming system)
Genetic basis for Resistance (direct and indirect defenses) and Adaptation

Agroecology

■ Scientific context

- Better used of biodiversity and biological regulations in agroecosystems to reduced chemical inputs



- Link the microbial biodiversity and the plant ecosystem

Importance of biodiversity for ‘natural’ ecosystem functioning



Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 1463–1468, February 1999
Ecology

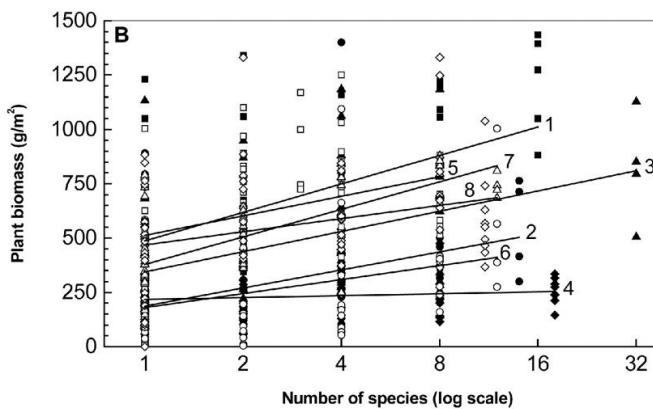
Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis

(stochastic dynamic model/species richness/ecosystem processes/temporal variability/ecosystem stability)

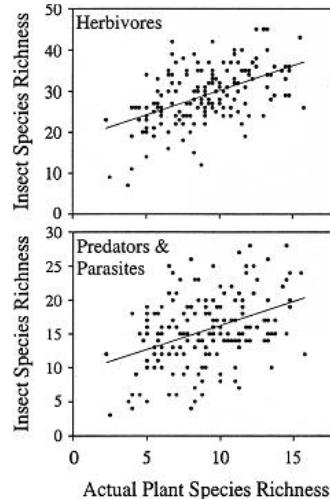
SHIGEO YACHI AND MICHEL LOREAU*

- functional redundancy
- resistance to perturbation
- resilience to damage
- functional disability
- susceptibility to perturbation
- lower resilience to damage

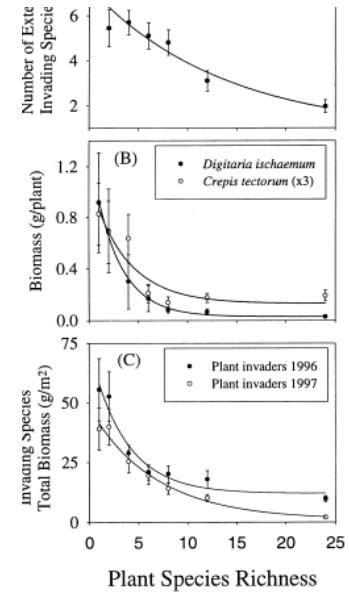
Diversity and Productivity in Long-Term Grassland Experiment: the lessons of the BIODEPTH experiment)



Loreau et al., Science 294:804-808 (2001)
 Tilman et al., Science 294:843-845 (2001)



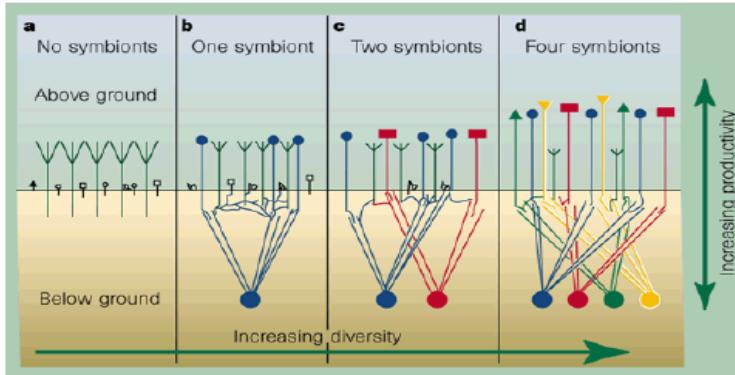
Adapted from Sieman et al.,
 The American Naturalist
 52: 738-750 (1998)



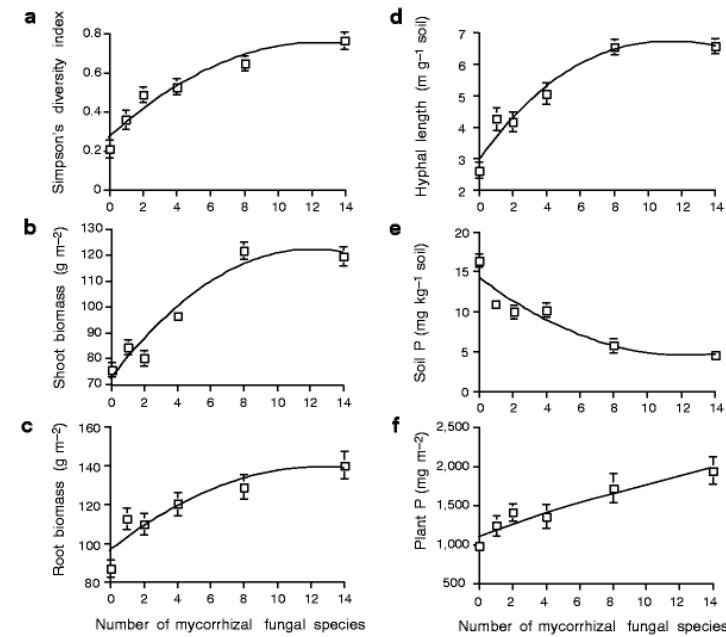
Knops et al., Ecology Letters
 2:286-293 (1999)

- High plant diversity is related to high ecosystem productivity
- a greater herbivores species richness (generalists + specialists) but also more natural enemies of these herbivores
- a greater resistance to biological invasion

Above and belowground diversity relationship: back to the root, case of root symbiosis

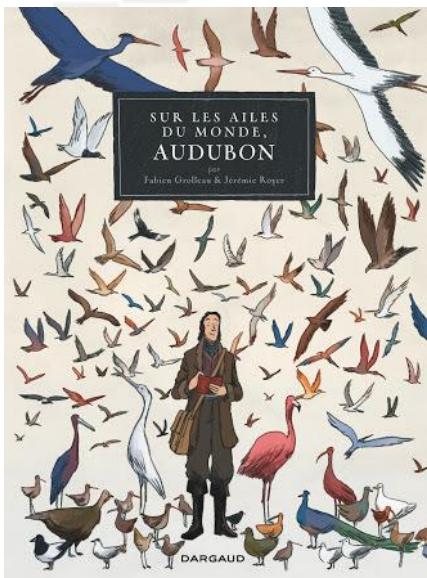


Van der Heijden et al., Nature 396:69-72 (1998)



- High mycorrhiza diversity is related to an increased of plant fitness by optimizing the resource utilization

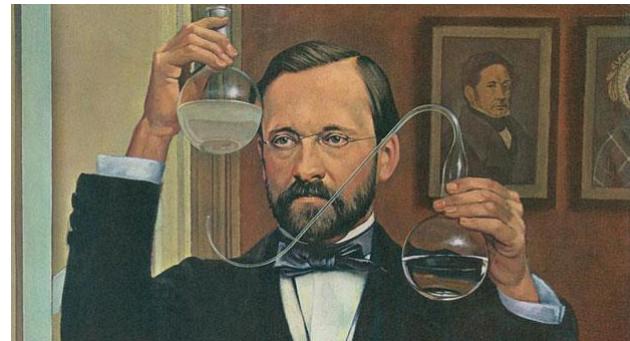
Ecology versus microbial ecology



Audubon (1785-1851)



Bonnier (1853-1922)



Pasteur (1822-1895)

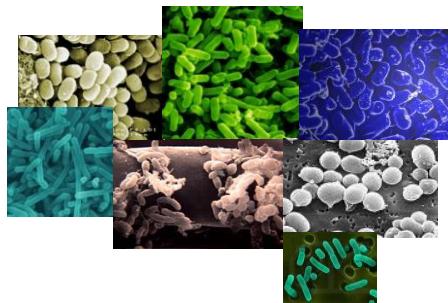


Microbiome

(2005-)

Huge number for microorganisms in open ecosystems

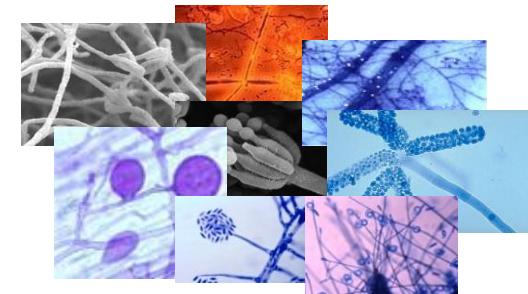
1 g of soil



1 milliard of bacteria
1 million species



1.5 tons/ha

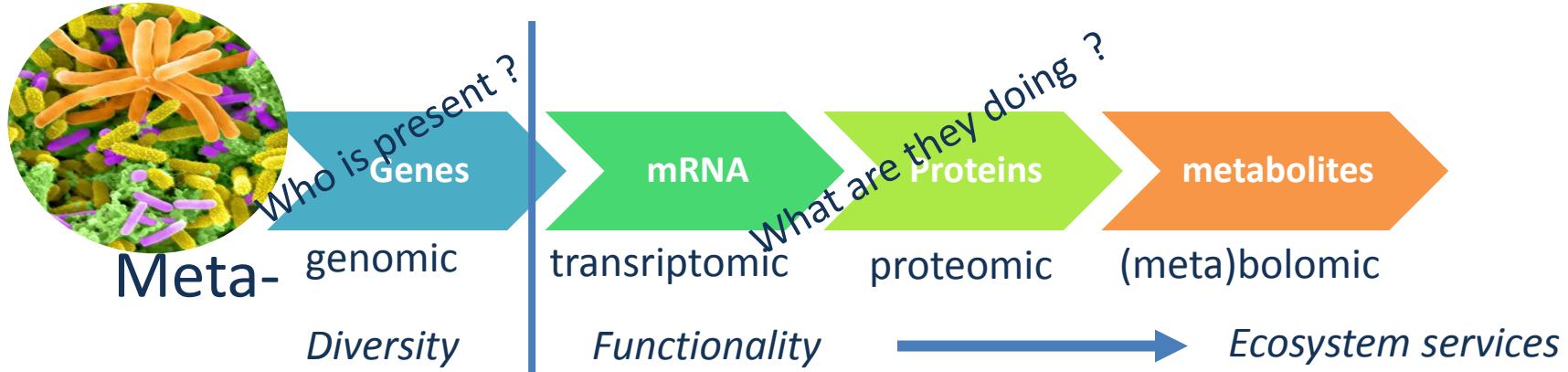


1 million of fungi
1000 species



3.5 tons / ha

Link community assembly and microbial functions in relation to plant ecosystem functioning



- Diversity analysis aimed to identify who are the microbial species (bacteria, dikarya, oomycetes,... and viruses) present and potentially the key players.
- Access to more functional markers (mRNA, metabolites, activity) remain a challenge for complex 'open' ecosystem => **complementary approaches between naturalistic, simplify ecosystem with diversity manipulation and synthetic microbial communities.**

Microbial diversity analysis (1/2)

➤ Biology

Soil / Root



SOP - Extraction

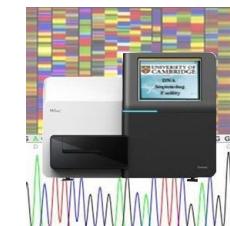
DNA/RNA



SOP – PCR
Multiplexing

HTS

MiSeq 2*300
(100000 reads)



GenoScreen

Terrat *et al.*, Microbial Biotech 8: 131-142(2015)

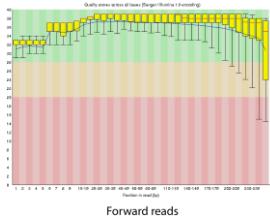
Terrat *et al.*, Microbial Biotech 5: 135-141 (2012)

Plassart *et al.*, PLOS One 7:e44279 (2012)

Microbial diversity analysis (2/2)

➤ Bioinformatic

Raw reads



Clean reads

Denoising
Trimming

Workflow GnS-PIPE

Dereplication
Clustering
Alignment
Taxonomy

Count tables

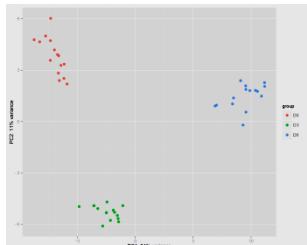
Index

- Richness
- Diversity (Shanon,...)
- Equitability

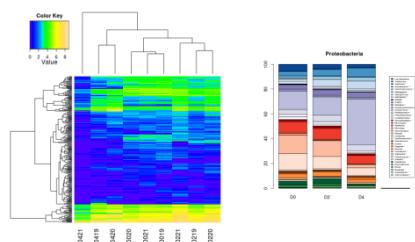
➤ Statistics (coll J. Aubert)



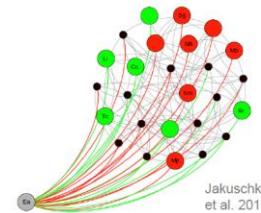
Classification



Discriminant analysis

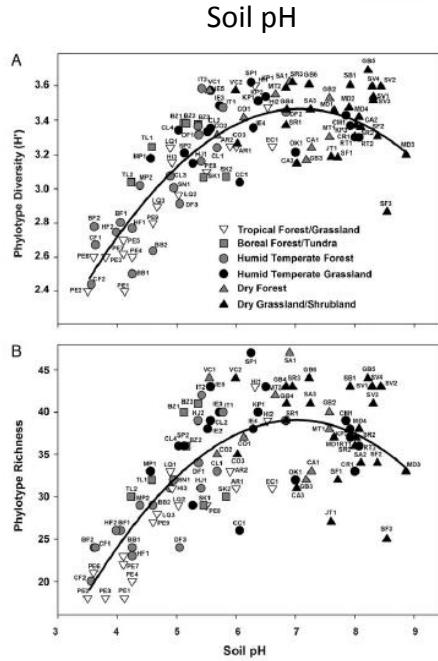


'Networks'



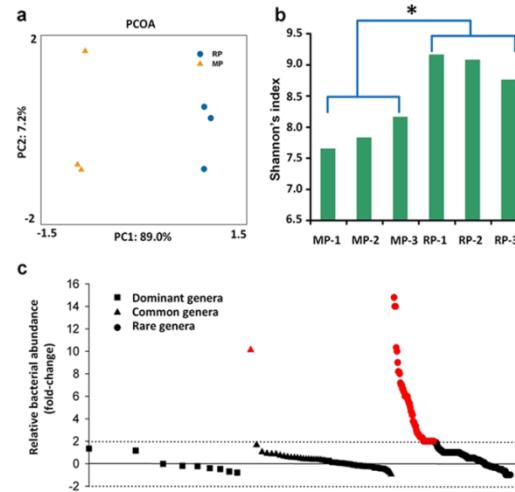
**'new methods:
Bi-clustering'**

Diversity reservoir = soil



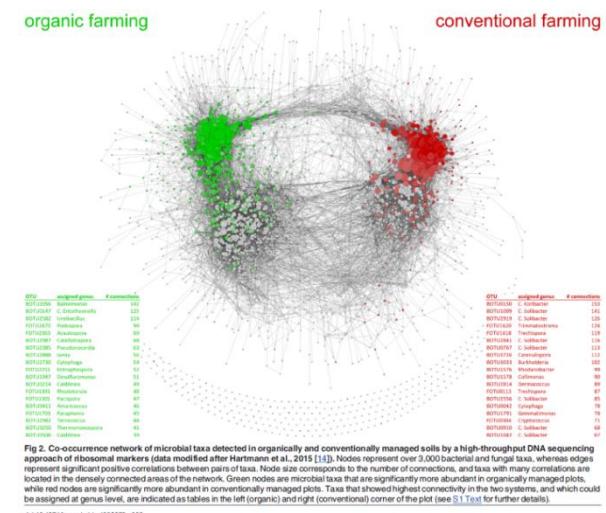
Fierer et Jackson, PNAS 2006

Monoculture versus Rotation



Li et al. ISME Journal 2018

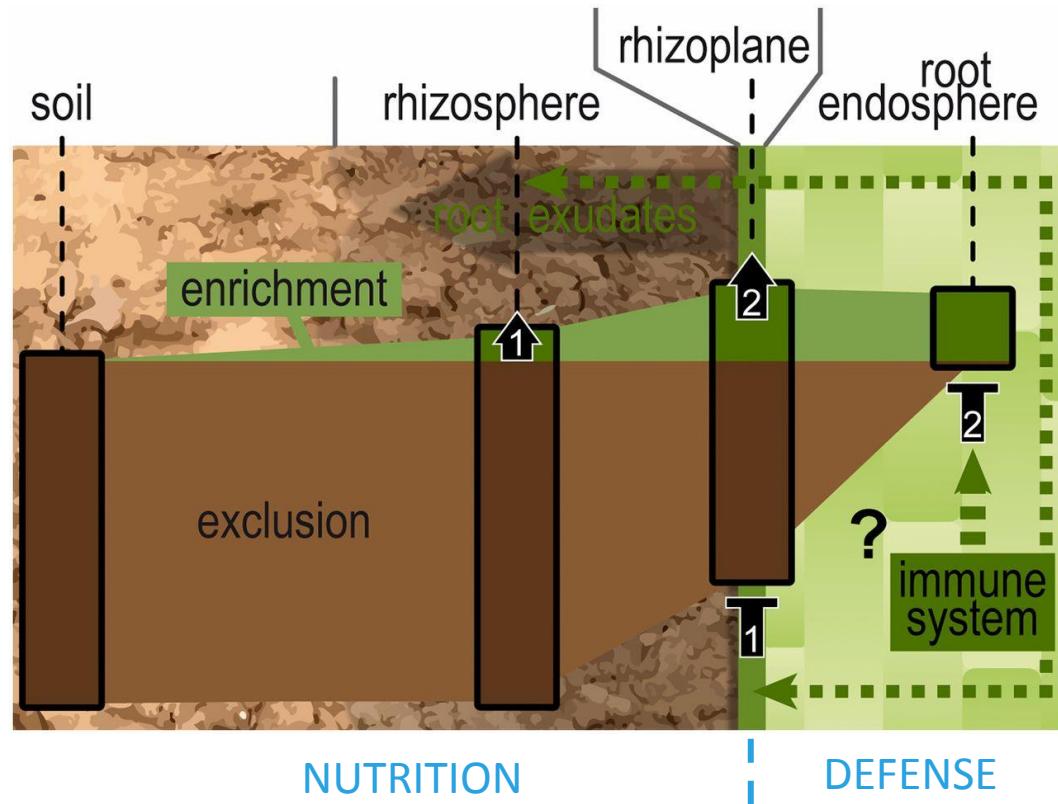
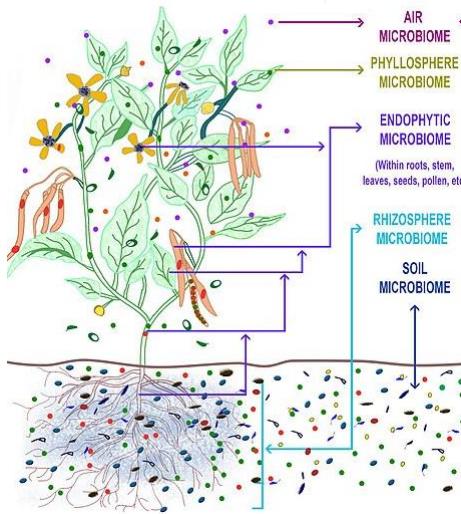
Conventional versus organic farming



van der Heijden & Hartmann PLOS Biol 2016

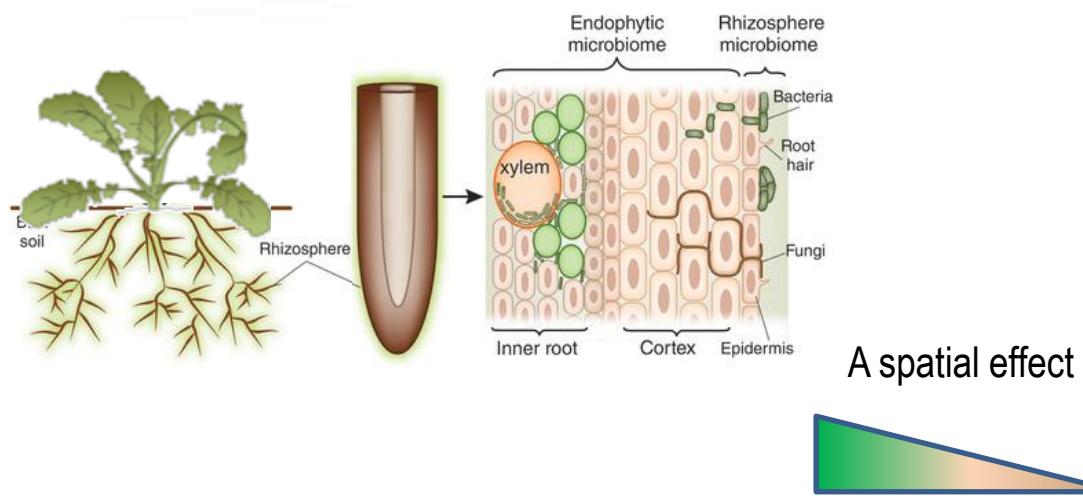
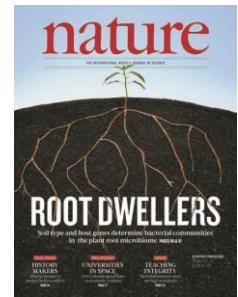
- Diversification forces: pedo-climatic (pH, texture, SOM) >> agronomic practices

Rhizosphere

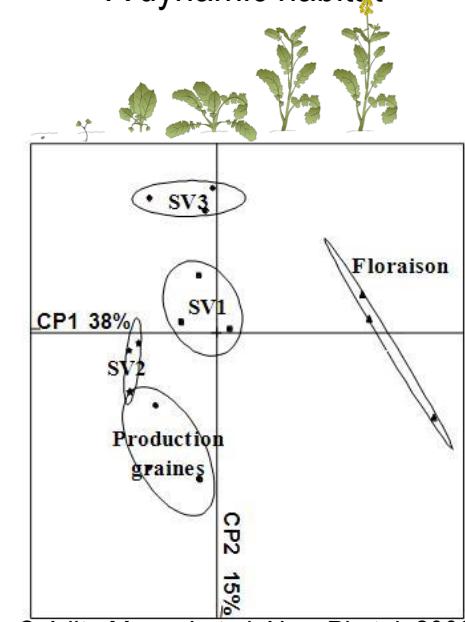


van der Heijden & Schlaepi 2015
<http://www.pnas.org/cgi/doi/10.1073/pnas.1500709112>

The rhizosphere: a spatialized habitat and a temporal dynamics



A dynamic habitat

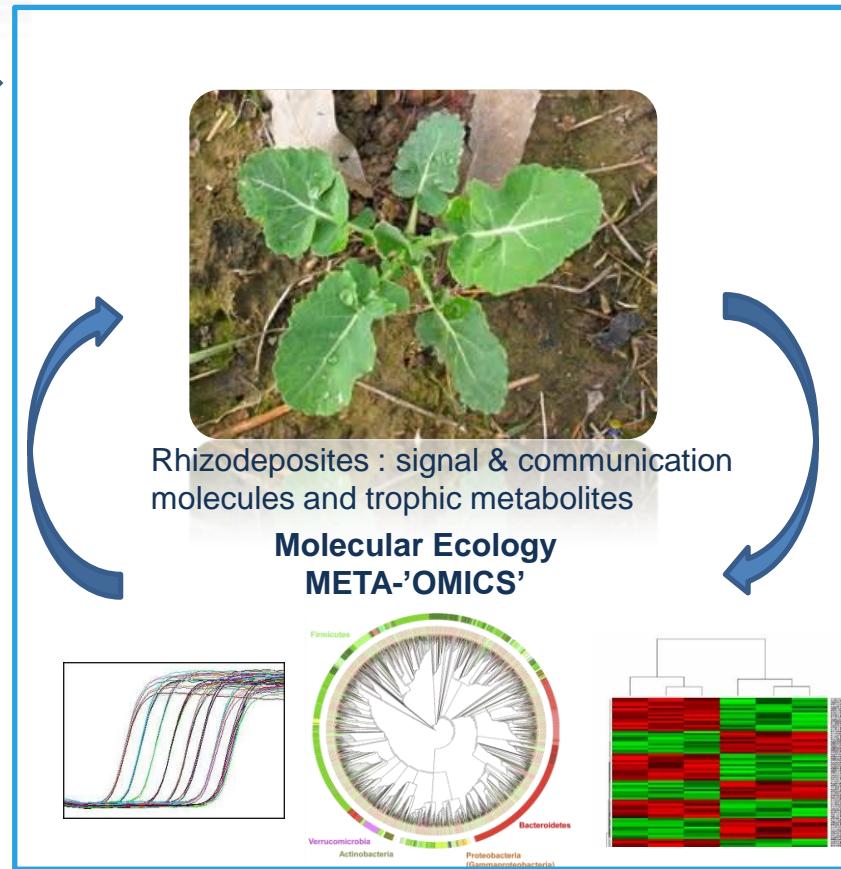


Credit : Mougel et al. New Phytol. 2006

Feed back loop in plant - microbiote interactions

Light
CO₂
eg Environment factors

Microbial communities effect on plant growth and health
=
Extended phenotype



Hologenome =
Plant genotype x
Micobial metagenome

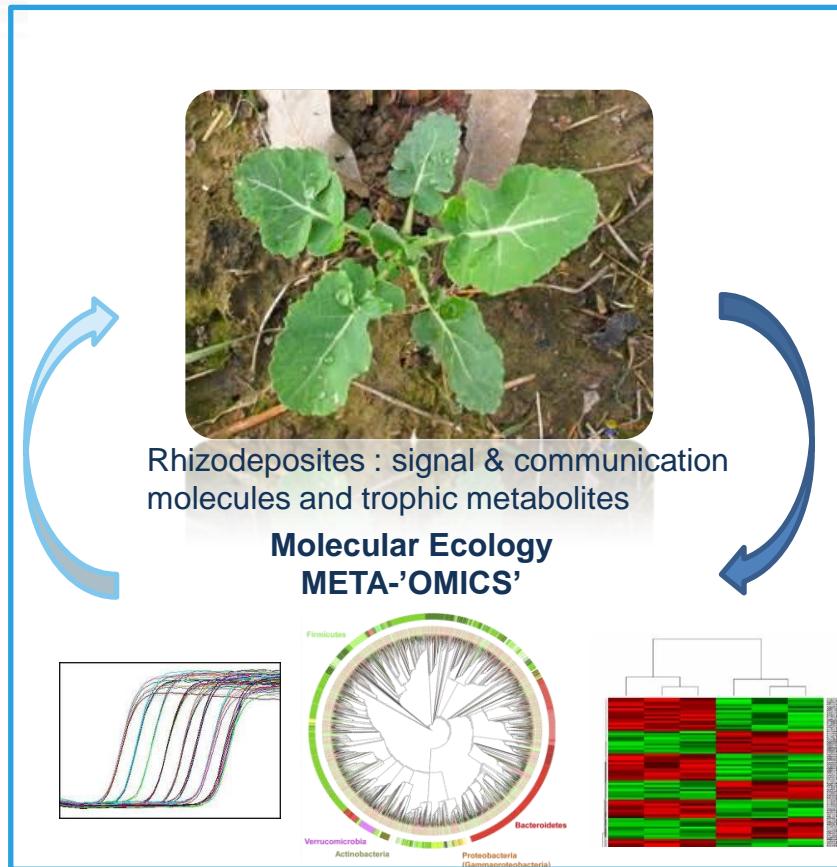
Plant effect on
microbial communities
ecology
=
Rhizosphere effect

Key question : understanding the link between microbial communities structure and functioning in relation to plant adaptation to biotic and abiotics factors.

Feed back loop in plant - microbiote interactions

Light
CO₂
eg Environment factors

Microbial communities effect on plant growth and health
=
Extended phenotype



Hologenome =
Plant genotype x
Micobial metagenome

Plant effect on
microbial communities ecology
=
Rhizosphere effect

Plant-microbial communities interactions

Host genetics: model plant
< 8 genotypes + related species

Environment



- Important factor = soil type (seed bank effect)
- Spatial effect: bulk soil < soil surrounding root system (ie rhizosphere) < endophytes
- Dynamic effect depending of plant phenology (vegetative versus reproductive stages)
- Core-microbiote with quantitative variations

Mougel et al., New Phytologist, 2006

Lundberg et al., Nature, 2012

Bulgarelli et al., Nature, 2012

Schlaepi et al., PNAS, 2013

Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana* relatives

Klaus Schlaepi^{a,b}, Nina Dombrowski^b, Ruben Garrido Oter^{a,c,d}, Emiel Ver Loren van Themaat^a, and Paul Schulze-Lefer^{a,d}

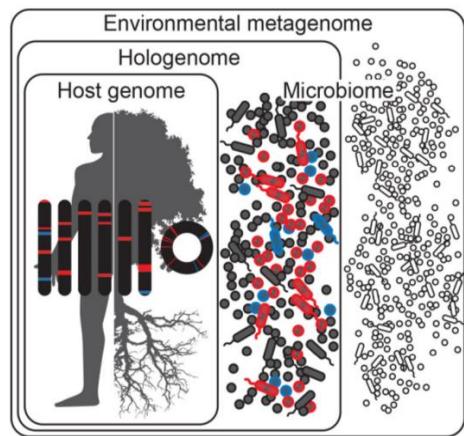
^aDepartment of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany; ^bPlant-Soil-Interactions, Institute for Sustainability Sciences, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland; ^cDepartment of Algorithmic Bioinformatics, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany; and ^dCluster of Excellence on Plant Sciences (CEPLAS), Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

This contribution is part of the special series of *PNAS* articles by members of the National Academy of Sciences elected in 2010.

PNAS

A conceptual framework: hologenome

Getting the Hologenome Concept Right:
an Eco-Evolutionary Framework for
Hosts and Their Microbiomes



Theis et al. *mSystems* 2016

$$P = G + E + (G \times E)$$

Environmental Microbiote (E)

$$P = G + E + (G \times E)$$

Host associated Microbiote (G)

- Evolutive perspectives
- Vertical versus horizontal transmission
- Consequences on host phenotype = extended phenotype

Holobiont concept



Aphid/
parasitoid



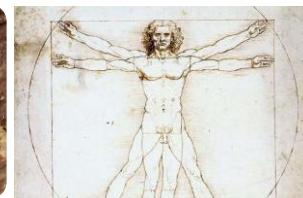
Fly/
parasitoid



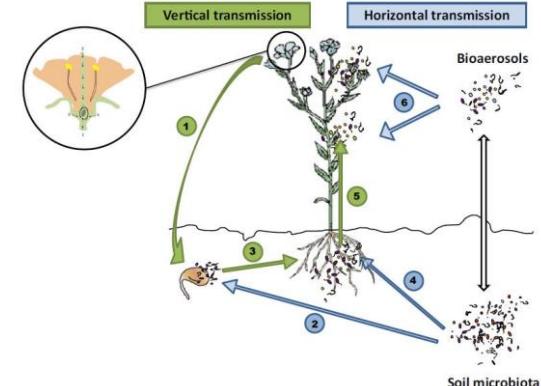
Bee



Termite



Human



Plant

- seed (cf. travaux M Barret)
- *Festuca* sp. / *Lolium* sp and fungi *Neotyphodium* sp.

Vertical transmission

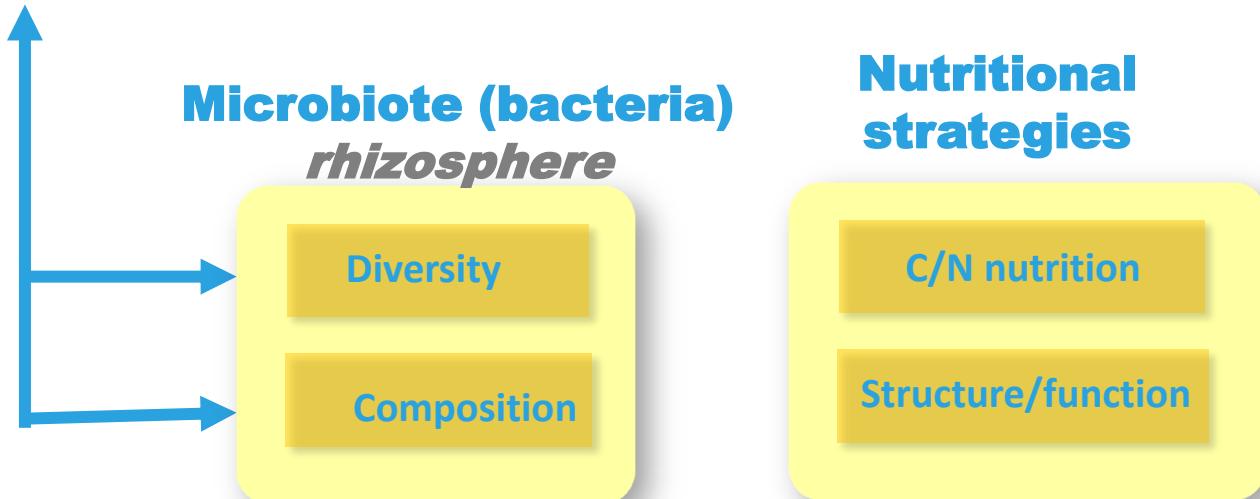
Vertical/horizontal transmission

A genome wide analysis study GWAS to identify key plant genes

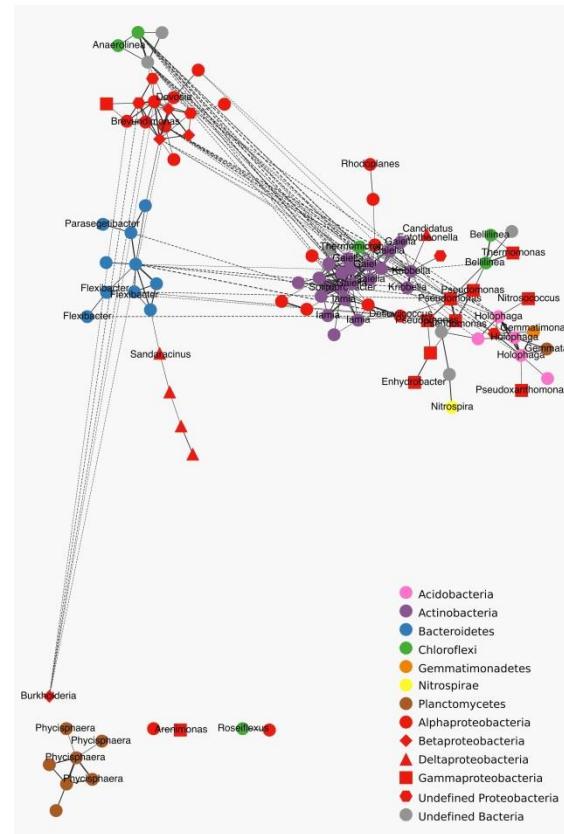
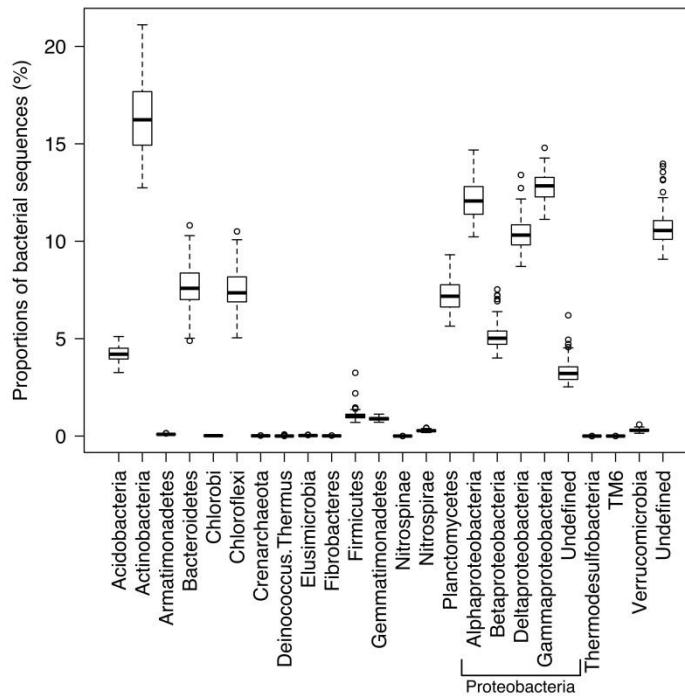
Host genotype

192 accessions of *M. truncatula* (core collection)

Environment
Green house



Microbiote analysis

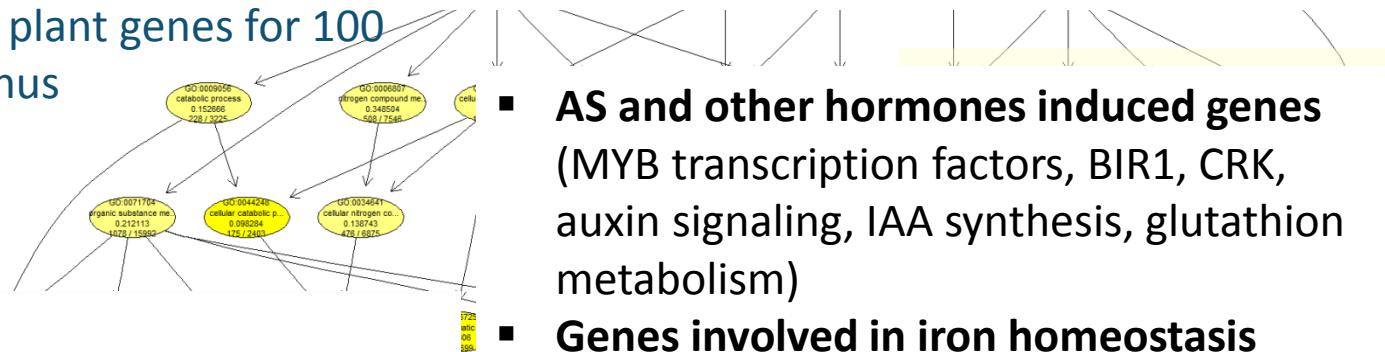


- Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes
- 157 OTU (core microbiote) with different count, share a significant heritability values (between 0,2 and 0,6)
- Co-occurrence between Proteobacteria-Acidobacteria and Proteobacteria-Actinobacteria-Bacteroidetes-Chloroflexi

Global analysis of significant plant genes related to dominant microbial ‘species’

✓ Singular Enrichment Analysis (Classic Fisher test,

TopGO R) : list of 2000 plant genes for 100 dominant bacterial genus



- AS and other hormones induced genes (MYB transcription factors, BIR1, CRK, auxin signaling, IAA synthesis, glutathion metabolism)
- Genes involved in iron homeostasis
- Translocation of carbon
- Nitrogen nutrition
- Phenylpropanoid metabolism

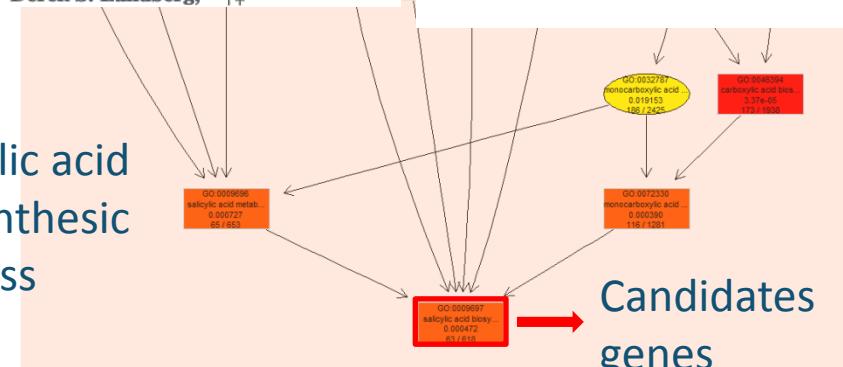
PLANT MICROBIOME

Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa

Science, 2015

Sarah L. Lebeis,^{1,2,*†} Sur Herrera Paredes,^{2,3,4,†} Derek S. Lundberg,^{2,5,‡‡}

Salicylic acid biosynthetic process

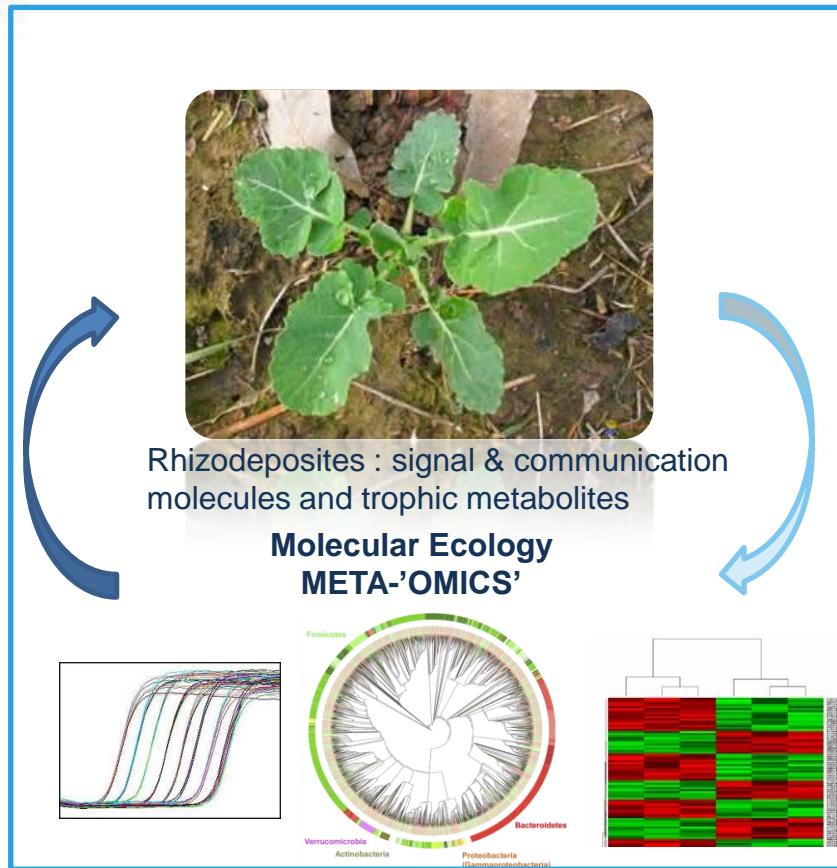


Plant defense

Feed back loop in plant - microbiote interactions

Light
CO₂
eg Environment factors

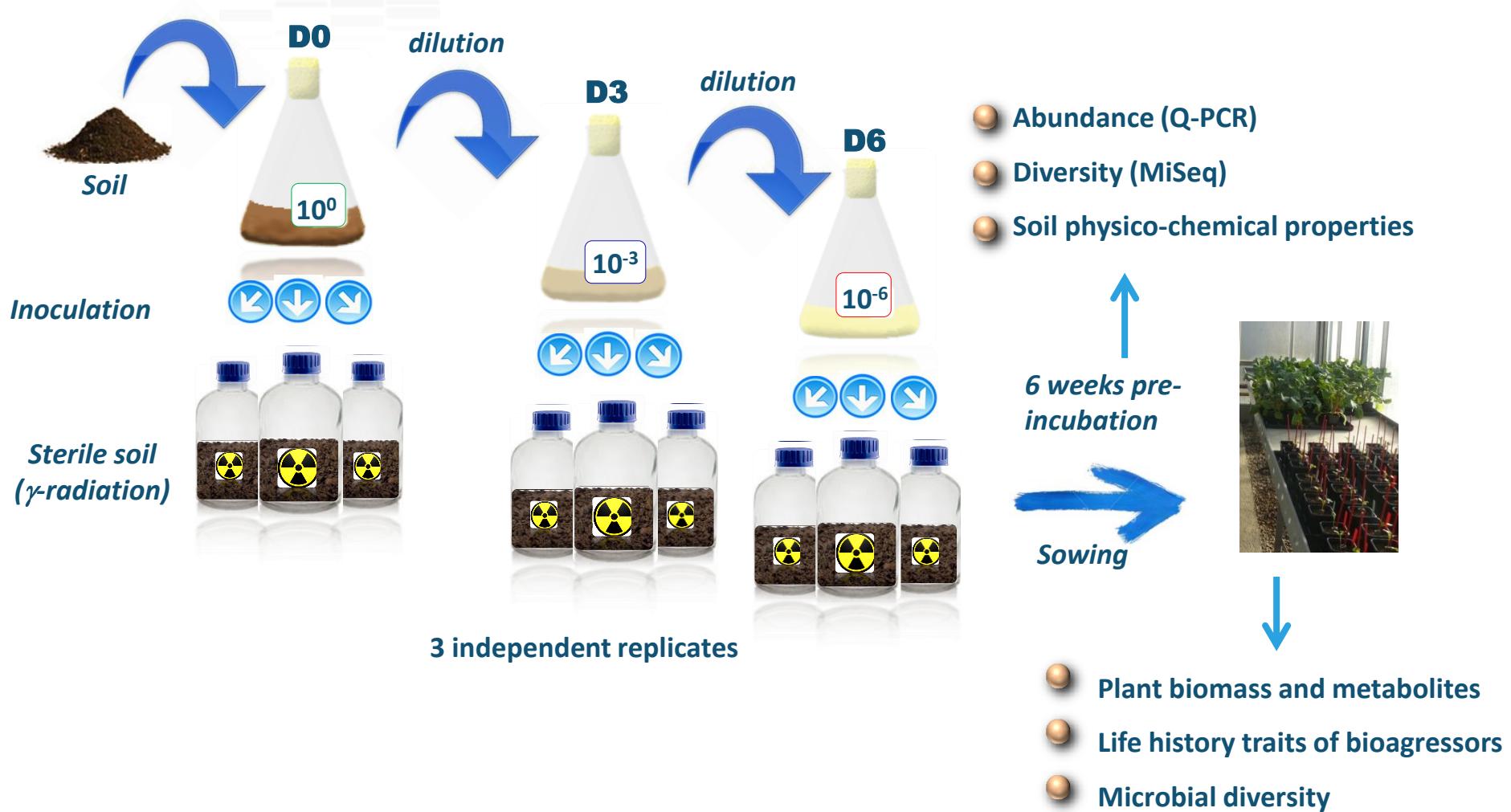
Microbial communities effect on plant growth and health
=
Extended phenotype



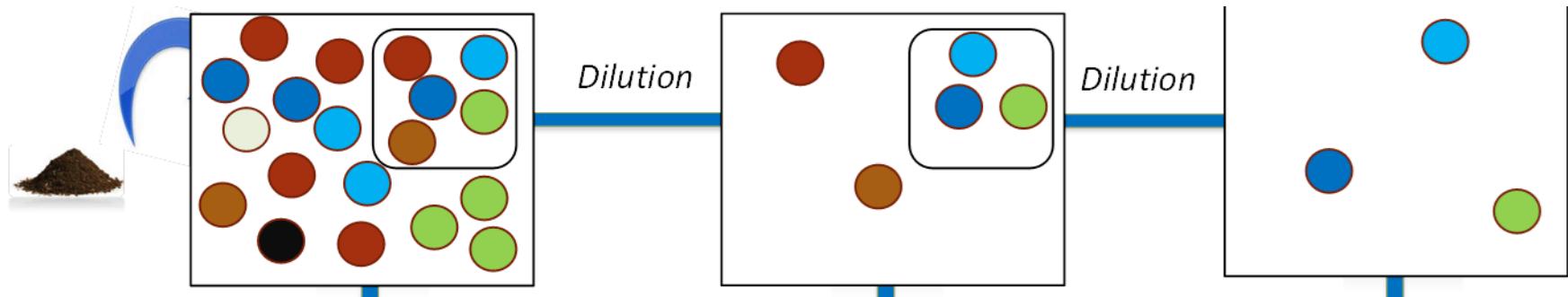
Hologenome =
Plant genotype x
Microbial
metagenome

Plant effect on
microbial communities
ecology
=
Rhizosphere effect

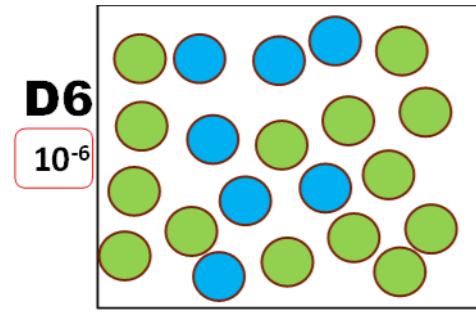
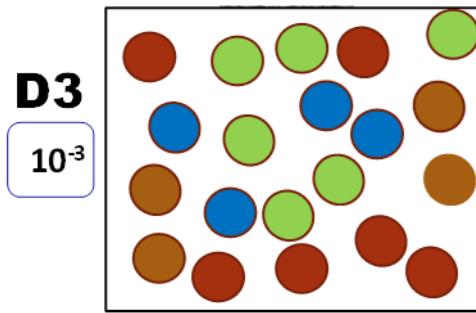
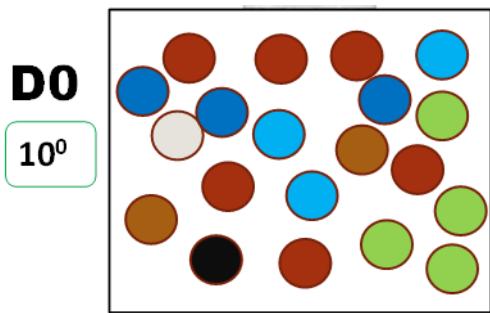
Microbial diversity manipulation : experimental diversity reduction



In theory: reduction of rare species and assembling of a new microbiote



➤ Sampling effect = reduction of rare species

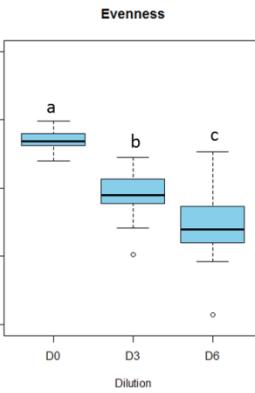
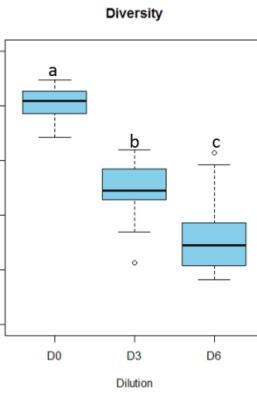
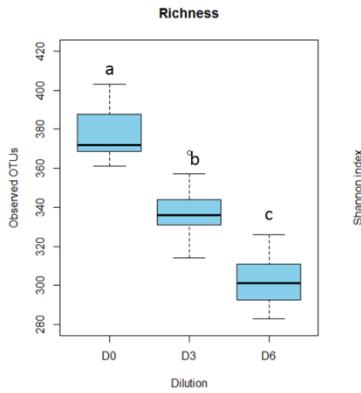


➤ Competition between species during colonization process (more competitive species, R strategy, copiotrophs)

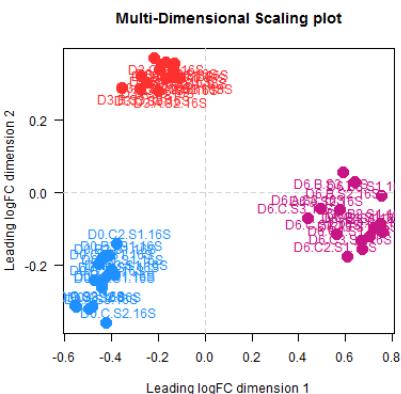
Microbial diversity manipulation : experimental diversity reduction

Bacteria

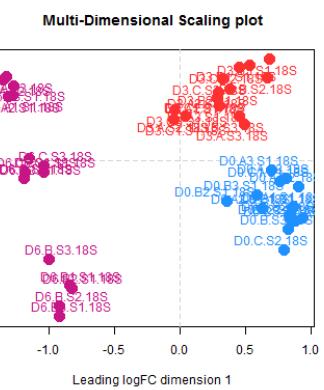
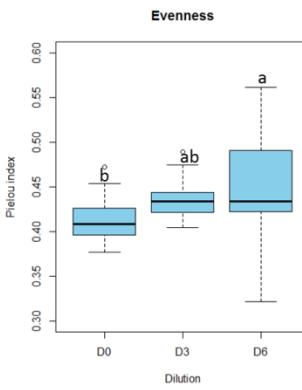
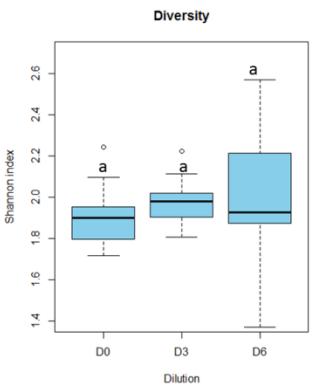
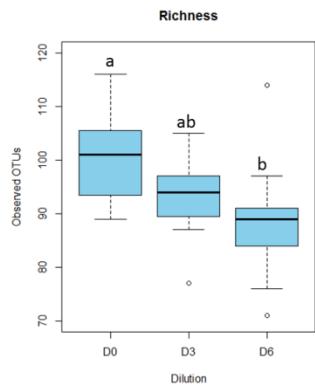
α diversity



β diversity



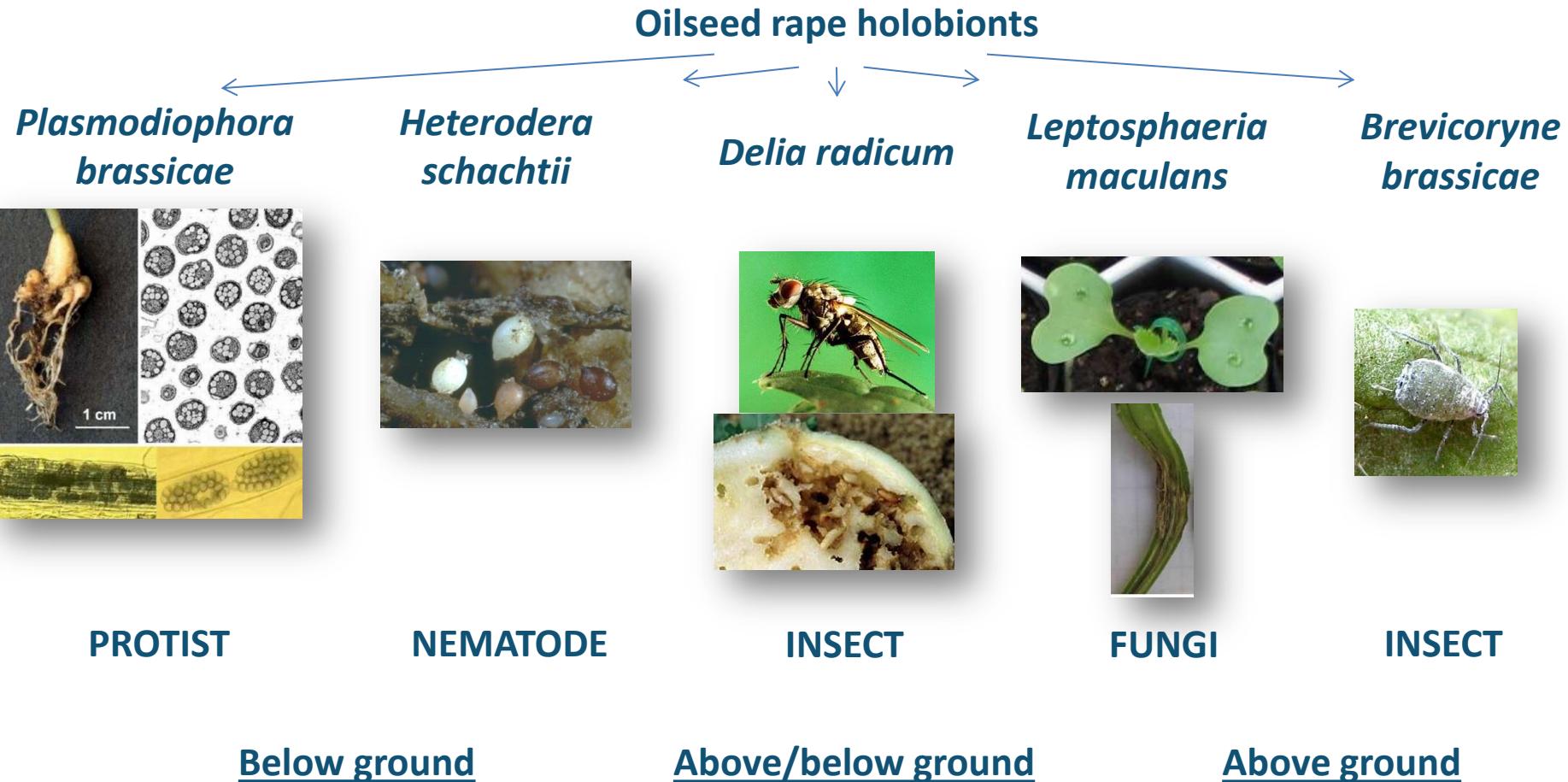
Fungi



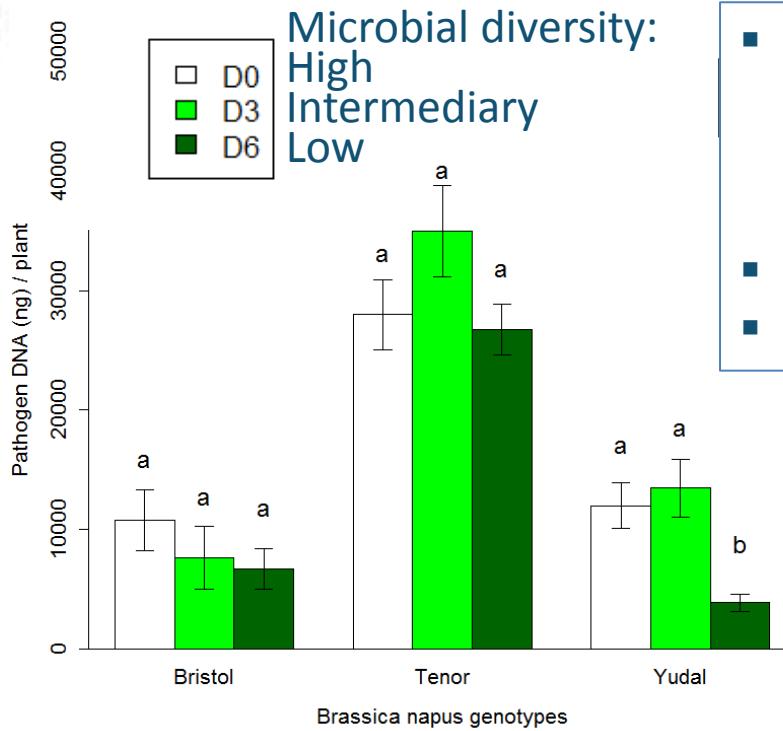
Richness (OTU numbers), Diversity (Shannon index) and Evenness (Piélou index)

MDS on distance matrix (Bray and Curtis distance)
(Axis 1= 34% and axis 2 = 11%)

Holobiont – bioaggressor interactions : a proof of concept



Plasmodiophora brassicaceae

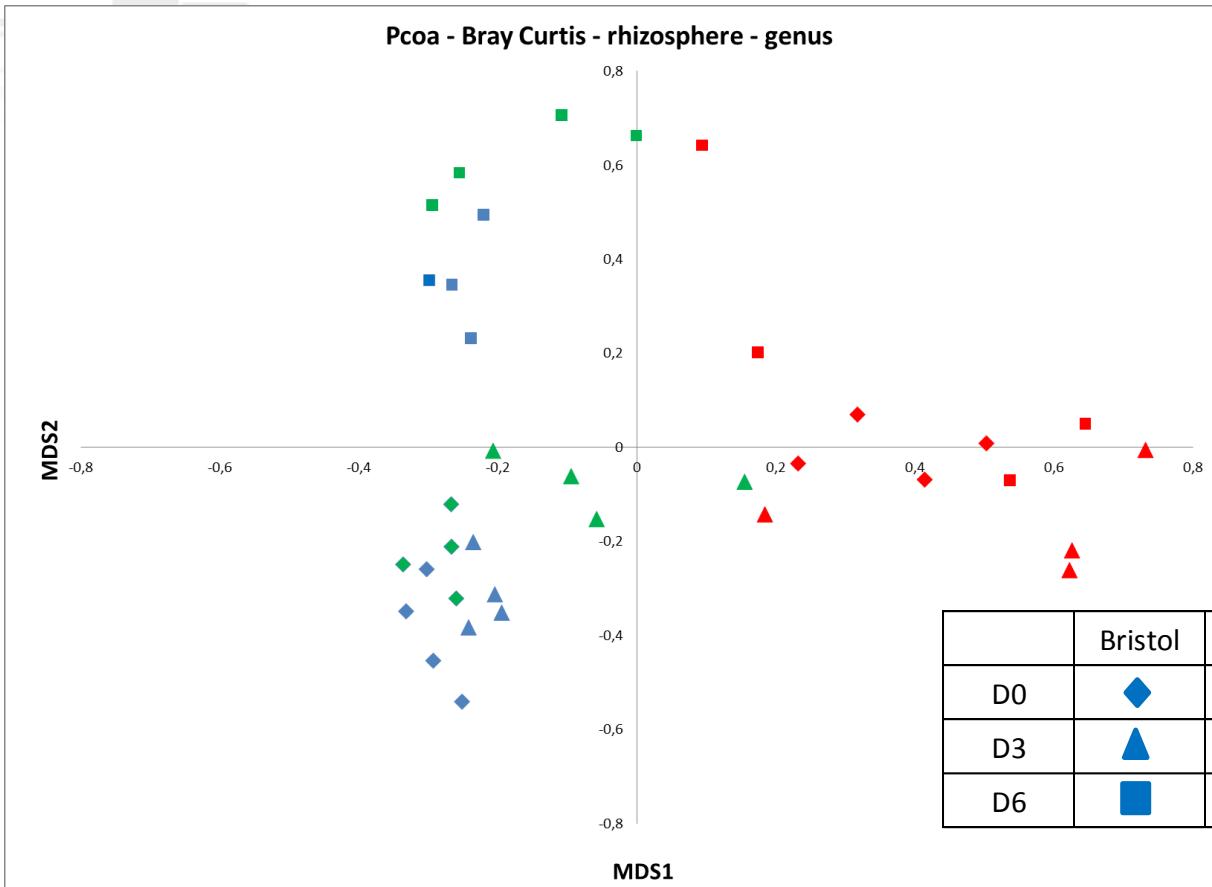


- 3 plant genotypes
Ténor = sensible
Yudal = tolerant
Bristol = resistant
- n=36 per dilution
- eH 10⁷ spores/plant

➤ the pathogen fitness (DNA amount) is affected by microbial diversity (on the tolerant plant genotype)

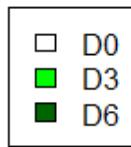


Modification of microbiote diversity



➤ Move from correlation to causation : functional analysis thanks to metatranscriptomics analysis of the holobiont in relation with pathogen (genomic data available)

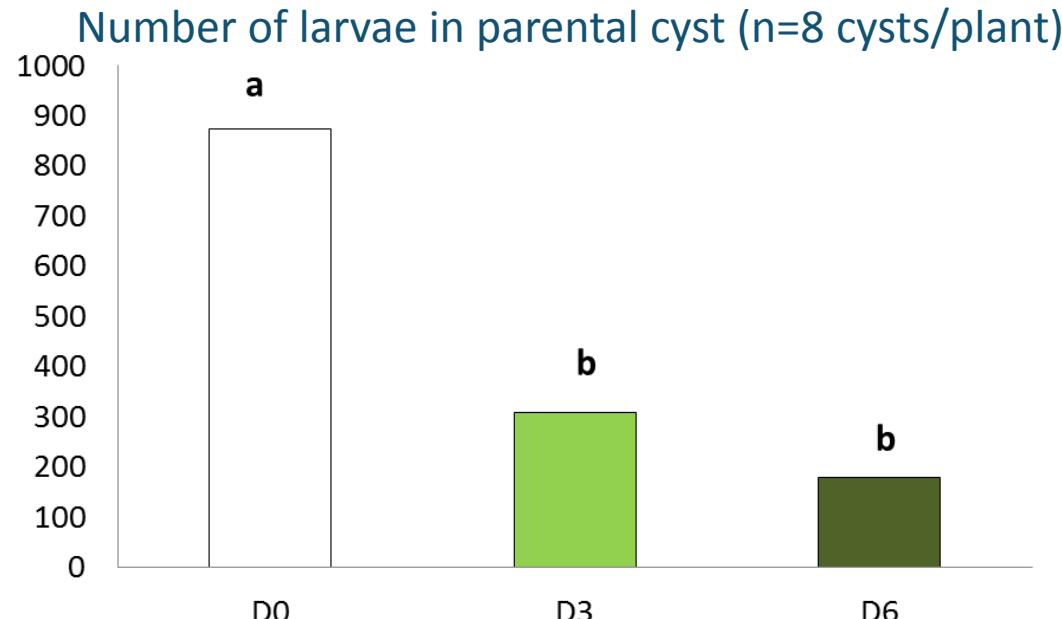
Heterodera schachtii



Microbial diversity:
High
Intermediary
Low



- 1 plant genotype = Ténor
- n=36 per dilution
- 8 cysts/plant

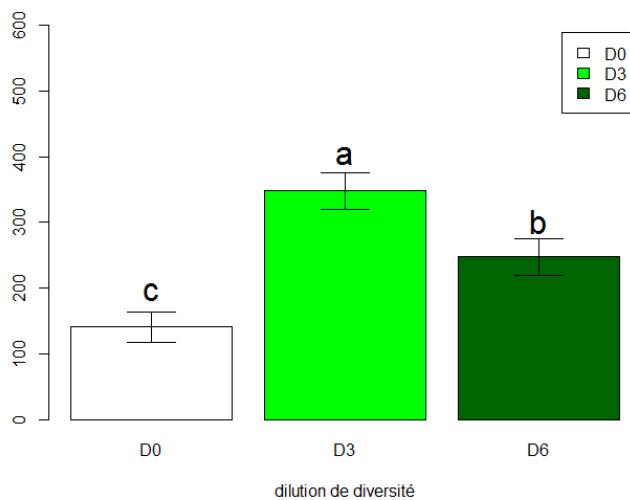


- female cysts hatching rate were affected by microbial diversity
- perspective : effect of holobiont root exudates (quantitative and qualitative analysis) on the cysts hatching kinetic (Coll Personeni & Cliquet EVA Caen)

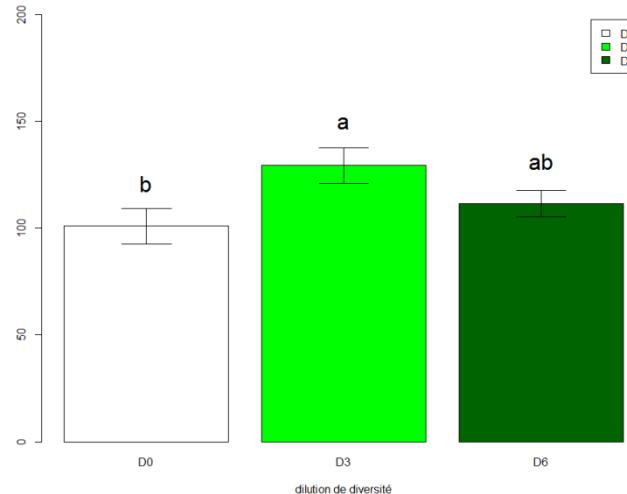
Heterodera schachtii



Number of cysts formed



Number of larvae / cyst (n=10 cysts/plant)

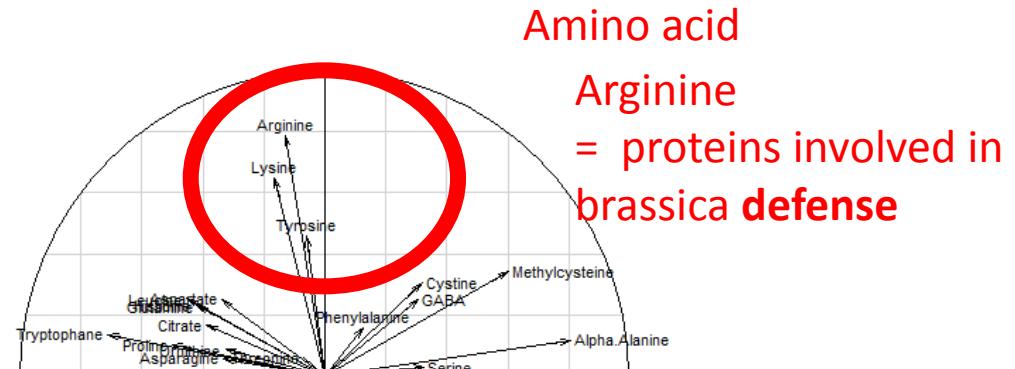
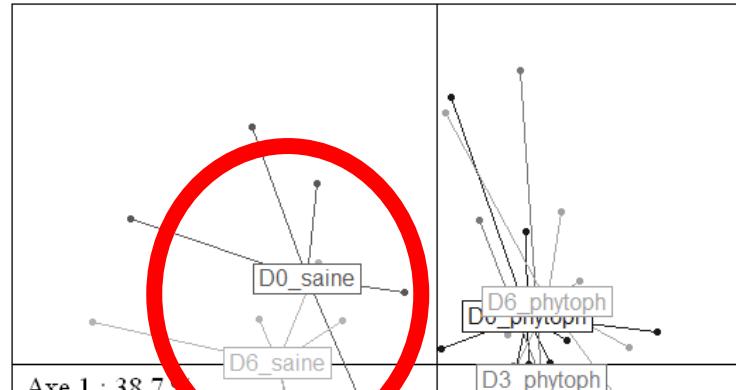


➤ ***Heterodera schachtii* fitness is affected by microbial diversity**

High diversity -> low hatching = low plant signals (exudates) perception

Low diversity -> high hatching but low cyst number = antagonism or competition with microbiota

Delia radicum - plant metabolites (primary and specific Brassica metabolites)



- the microbial diversity modified plant metabolites before insect infestation
- in progress : metatranscriptomics analysis of the different holobiont
- perspectives : *D. radicum* behaviours

(STATISTIC test= WILCOXON & KRUSKAL-WALLIS)

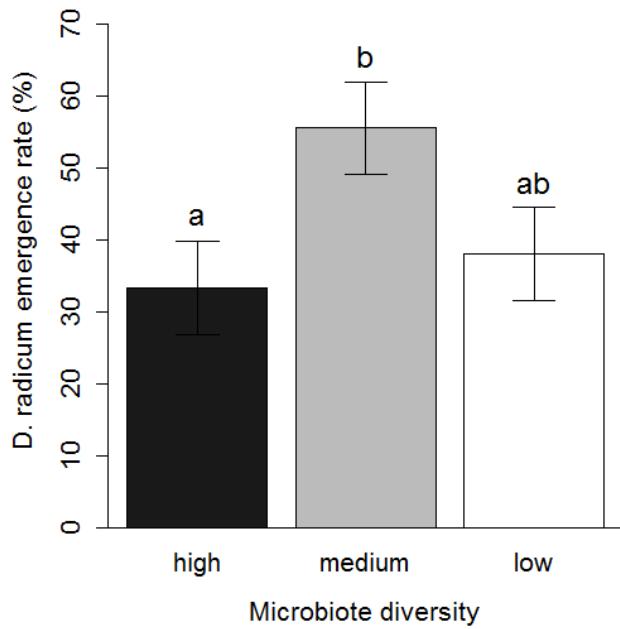
(Test statistique = PLS-DA)

Delia radicum

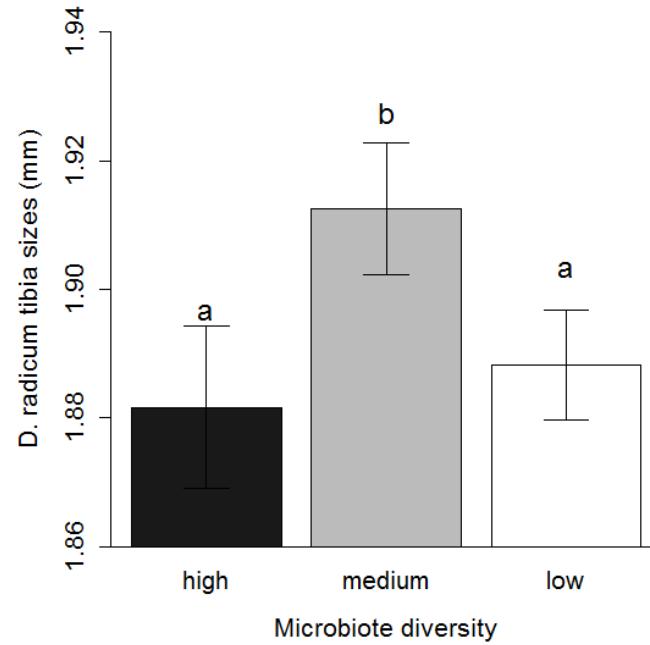
- Plant genotype : Ténor
- n= 18 per dilution
- 8 eggs/plant



Emergence rate



Tibia length

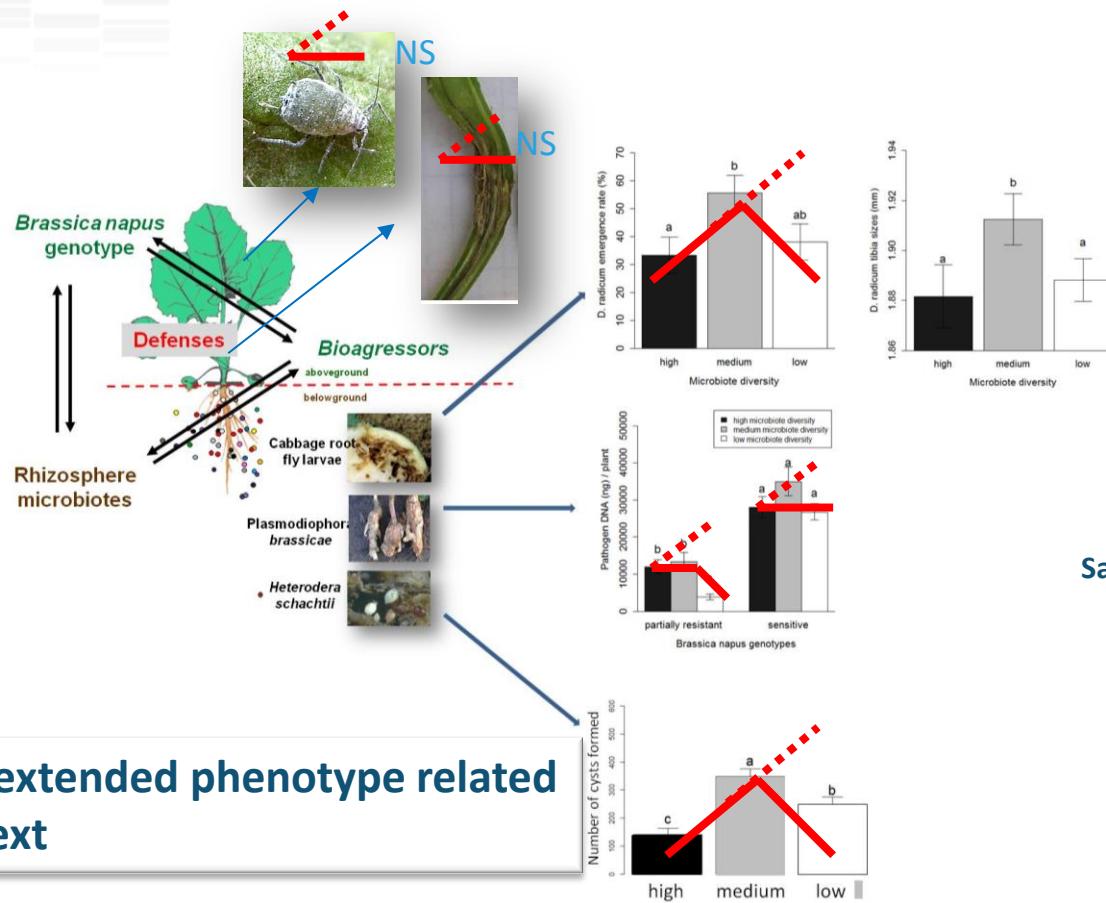


(Statistic test= Wilcoxon & Kruskal-Wallis)

➤ Emergence rate and the fitness of the root fly emergent were affected by microbial diversity (antagonism and/or competition)

Lachaise et al., Insect Sciences 2017

Summary



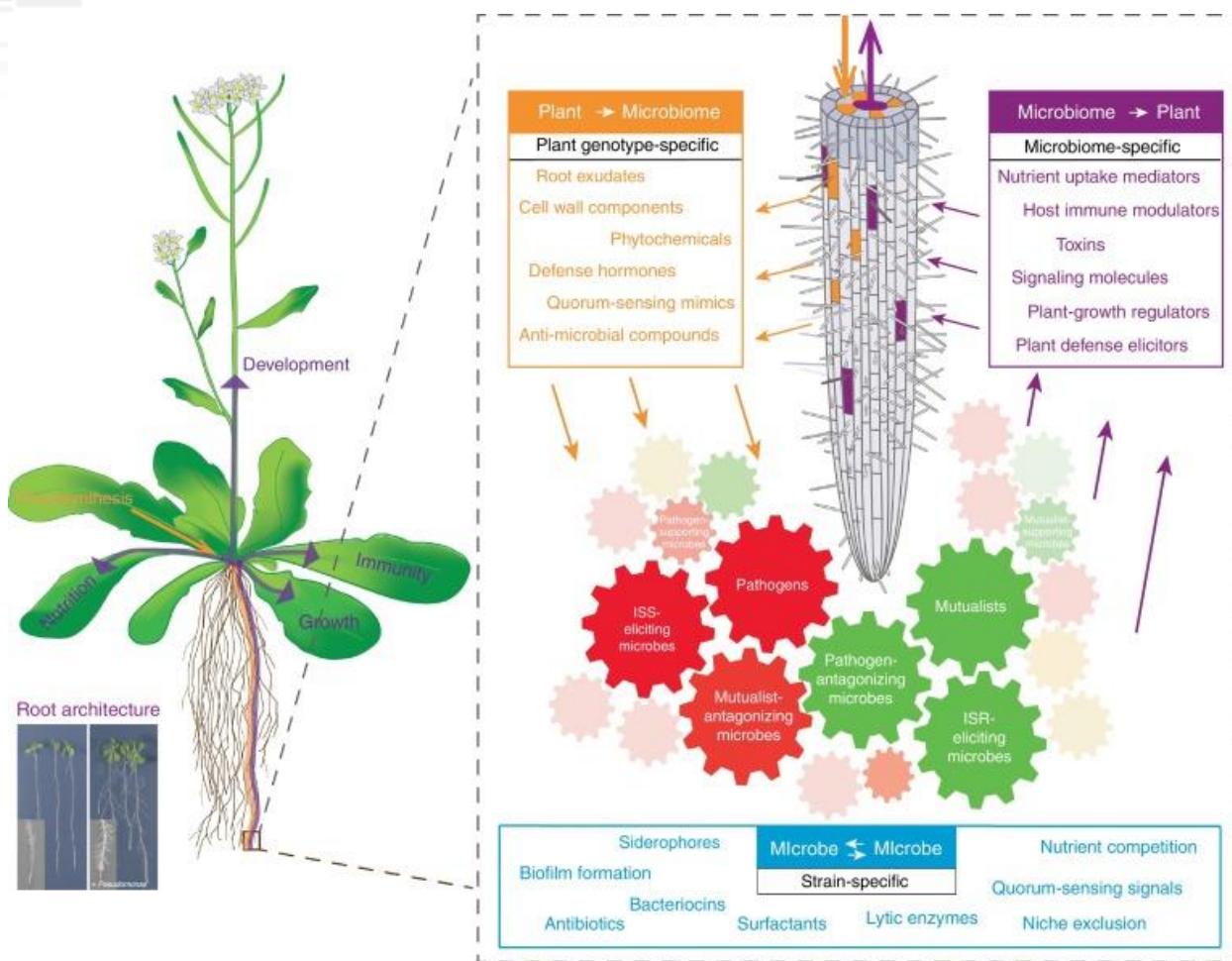
Lachaise et al., 2017
Orry et al., 2018

Lebreton et al. 2019
Sarniguet et al. in preparation

➤ Plant resistance extended phenotype related to microbiote context

Mougel et al. in preparation

Schematic view

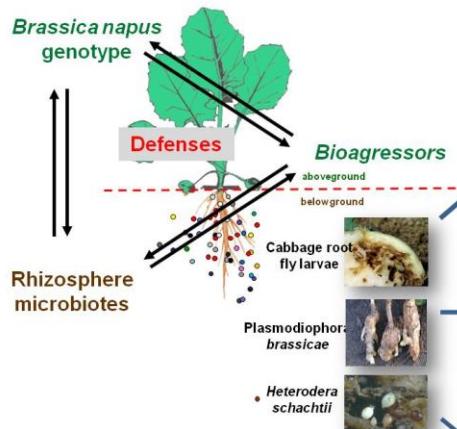


Trends in Plant Science

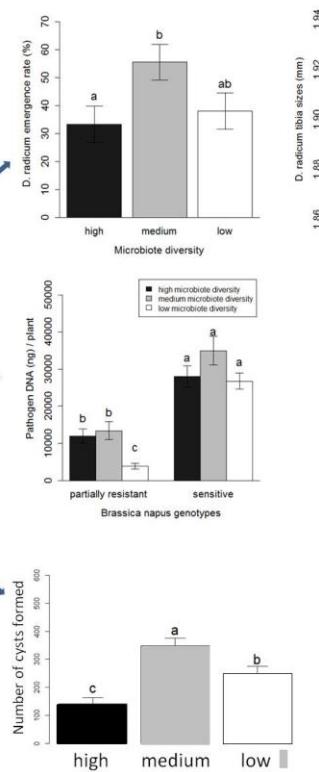
Pieterse CMJ et al., 2016

Next step (1/3): the triptic analysis

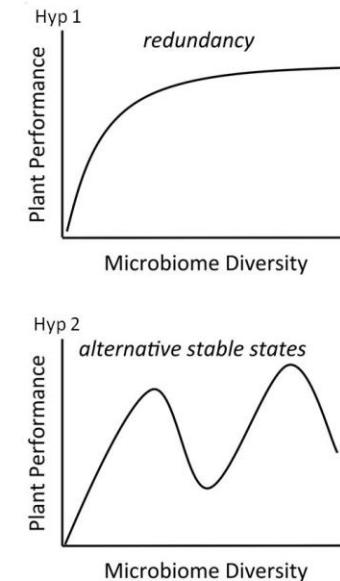
(A) MIRAGES project



(B) Effect of the resulting plant microbiota interaction on life history traits of three soil bioaggressors (Mougel et al., in preparation; Lachaise et al., in revision)



(C) Theoretical aspect. Adapted from de Naeem et al (2002)

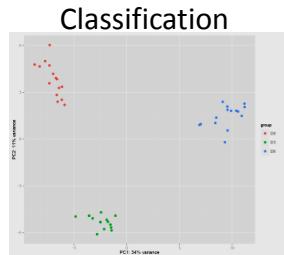


- Move from structure to functions (meta-transcriptomics, metabolomics)
- Importance of microbial interaction = go to the network
- Data integration (system ecology)

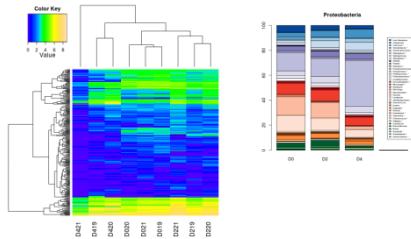
Next step (2/3): Networks: co-occurrence, ecological,...

Data available
Counting tables
(OTU, genes)
(and co-variables)

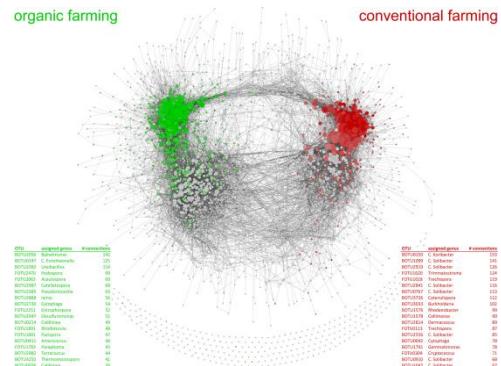
Diversity Index



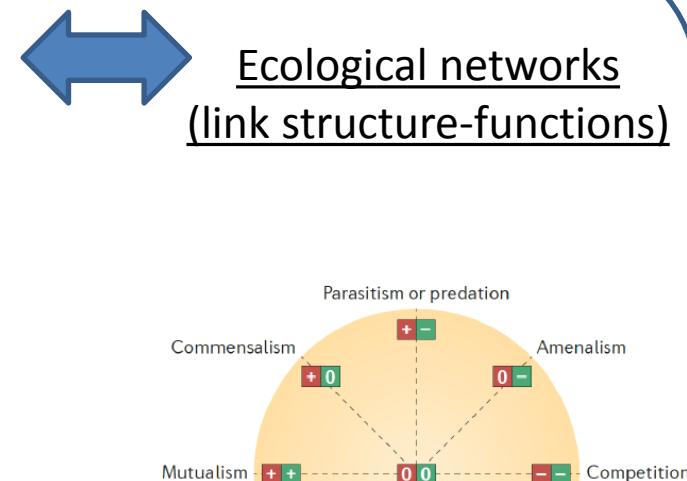
Classification



Network metrics/index
Co-occurrence networks
Genes network



Hartman et al., 2015;
Vacher et al., 2016)



- Better understanding of microbiote assembling, plant-microbiote interactions prediction, rule in plant performance, key members

Next step (3/3): Communities reconstitution experiments: synthetic microbial communities

- Classical approach for food/artificial digester ecosystems = selected ecosystem as starter in industrial processing (controlled inoculum)
- Need of cultivable microorganisms with genomic data
- Approach followed in complex environment (rhizosphere/phyllosphere): move to correlation to causation = system ecology/biology

OPEN  ACCESS Freely available online

PLOS | GENETICS

A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota

Natacha Bodenhausen¹, Miriam Bortfeld-Miller¹, Martin Ackermann^{2,3}, Julia A. Vorholt^{1*}

¹Institute of Microbiology, ETH Zurich, Zurich, Switzerland, ²Department of Environmental Sciences, ETH Zurich, Zurich, Switzerland, ³Department of Environmental Microbiology, Eawag, Dübendorf, Switzerland

Abstract
The identity of plant host genetic factors controlling the composition of the plant microbiota and the extent to which plant genes affect associated microbial populations is currently unknown. Here, we use a candidate gene approach to investigate the role of plant host genes in shaping the phyllosphere microbiota. We used a synthetic community approach to identify host mask genetic factors, the model plant *Arabidopsis thaliana* was used in a gnotobiotic system and inoculated with a reduced complexity synthetic bacterial community composed of seven strains representing the most abundant phyla in the phyllosphere. From a panel of 55 plant mutants with alterations in the surface structure, cell wall, defense signaling, secondary metabolism, and pathogen recognition, a small number of single host mutations displayed an altered microbiota composition and/or abundance. Host alleles that resulted in the strongest perturbation of the microbiota relative to the wild-type were *lacs2* and *pet1*. These mutants affect cuticle formation and led to changes in community composition and an increased bacterial abundance relative to the wild-type plants, suggesting that different bacteria can benefit from a modified cuticle to different extents. Moreover, *lacs2* and *pet1*, which are involved in ethylene signaling as key factor modulating the community's composition. Finally, we found that two different *Arabidopsis* accessions exhibited different communities, indicating that plant host genetic factors shape the associated microbiota, thus harboring significant potential for the identification of novel plant factors affecting the microbiota of the communities.

ARTICLE

doi:10.1371/journal.pgen.16192

Functional overlap of the *Arabidopsis* leaf and root microbiota

Yang Bai^{1*}, Daniel B. Müller^{2*}, Girish Srinivas^{3*}, Ruben Garrido-Oteo^{1,3,4#}, Eva Potthoff², Matthias Rott¹, Nina Dombrowski¹, Philipp C. Münch^{5,6,7}, Stijn Spaepen¹, Mitja Remus-Emsermann², Bruno Hütte⁸, Alice C. McHardy^{4,5}, Julia A. Vorholt^{1*} & Paul Schulze-Lefert^{1,4#}

Roots and leaves of healthy plants host taxonomically structured bacterial assemblies, and members of these communities contribute to plant growth and health. We established *Arabidopsis* leaf- and root-derived microbiota culture collections representing the majority of bacterial species that are reproducibly detectable by culture-independent community sequencing. We found an extensive taxonomic overlap between the leaf and root microbiota. Genome drafts of 400 isolates revealed a large overlap of genome-encoded functional capabilities between leaf- and root-derived bacteria with few significantly different features. Using a *gnotobiotic* approach, we show that *Arabidopsis* plants harbor a distinct and a gnotobiotic *Arabidopsis* plant system. We show that the isolates form assemblages resembling natural microbiota on their cognate host organs, but are also capable of ectopic leaf or root colonization. While this raises the possibility of reciprocal relocation between root and leaf microbiota members, genome information and recolonization experiments also provide evidence for microbiota specialization to their respective niche.

*A collaborative work ! Need of Math !
Thanks for your attention !*

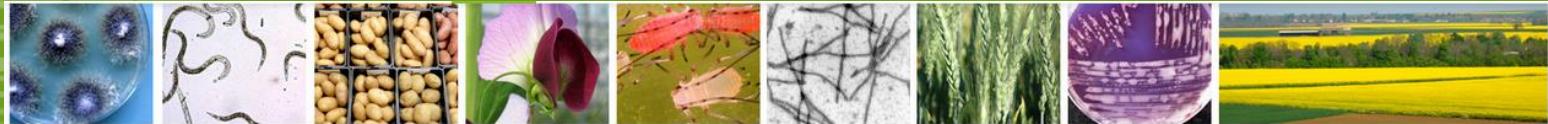
Using DNA metabarecoding to study plant-microbiote interactions

Genetic basis of plant-microbiote interactions using a GWAS approach

Alves-Carvalo S., Aubert J., Bousset L., Cortesero A.M., Daval S., Delourme R., Ermel M., Fournet S., Gazengel K., Gravot A., Guillerm-Erckelboudt A.Y., Lachaise T., Lebreton L., Linglin J., Luquet M., Manzanares-Dauleux M., Montarry J., Poinsot D., Porte C., Sarniguet A., Simon J.C.



Plant Health and Environment division
Microbial Ecosystem and Meta'Omics Metaprogram
ANR and Plant2Pro



Using DNA metabarcoding to study plant-microbiote interactions

Ch. Mougel: Genetic basis of plant-microbiote interactions using a GWAS approach

J. Aubert: Bioclustering via latent block model for overdispersed count data: application in microbial ecology



Christophe MOUGEL

Séminaire Math For Genomics
Evry 29 mai 2019