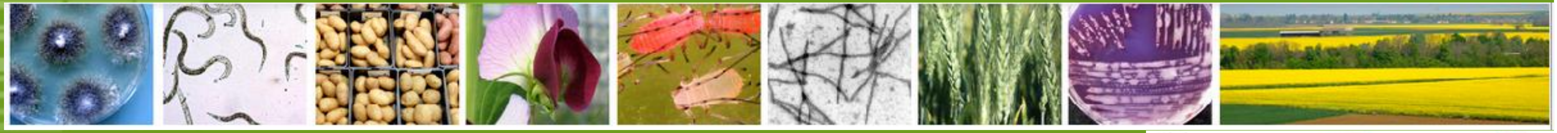
Institute for Genetics, Environment and Plant Protection - IGEPP



Team Resistance and Adapatation

Plant-microbial communities interactions : influence on plant health and growth

Christophe MOUGEL
christophe.mougel@inra.fr

Carrefour Recherche Plant de Pomme de Terre
November 15-16, 2016

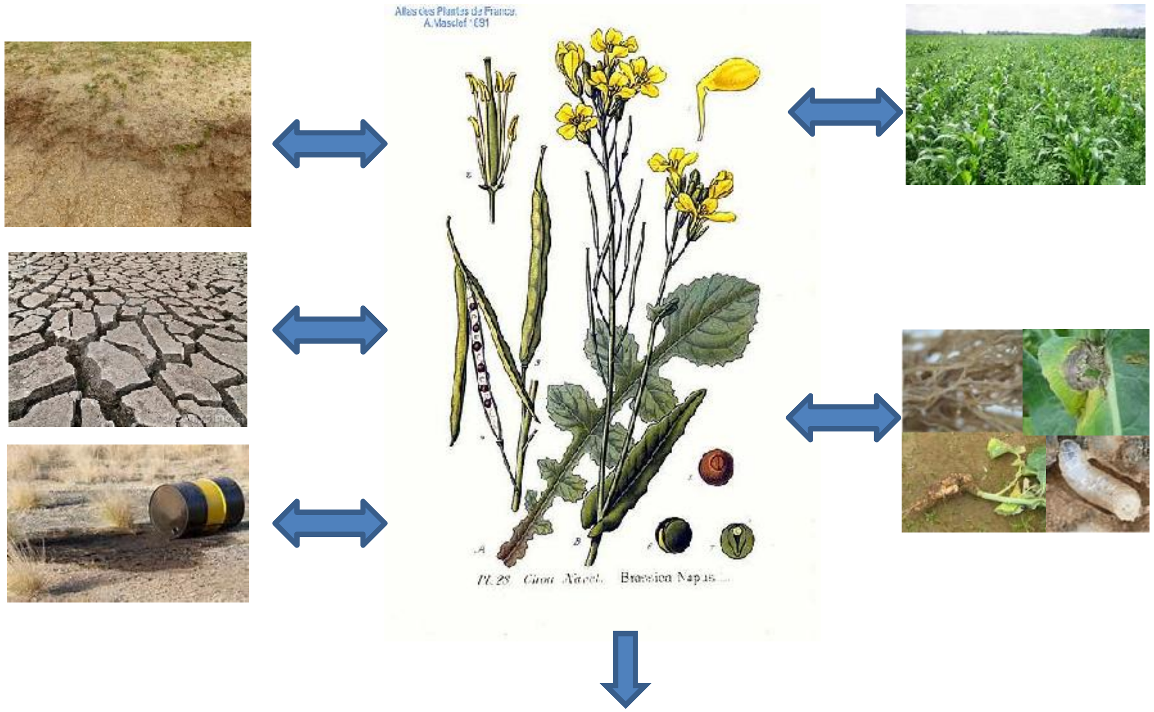
Agroecology

- Plant are under stesses

Abiotic stresses

Cultivated plant

Biotic stresses



Nutrition:
N, P, S

Climate change:
temperature, water

Pollution:
organic and heavy metals

Competition:
weeds

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

- Agriculture intensification

- Genetic basis for Resistance (direct and indirect defenses) and Adaptation

Agroecology

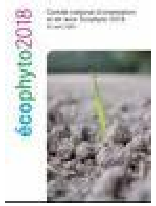
- Societal context
  - Conception of new agricultural practices

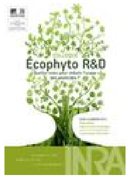
Maintain productivity

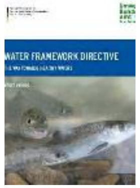
and reduced environmental impacts

- F - Grenelle environnement
- F - Ecophyto II 2025
- EU WaterFramework Directive

(waiting for those on soil)

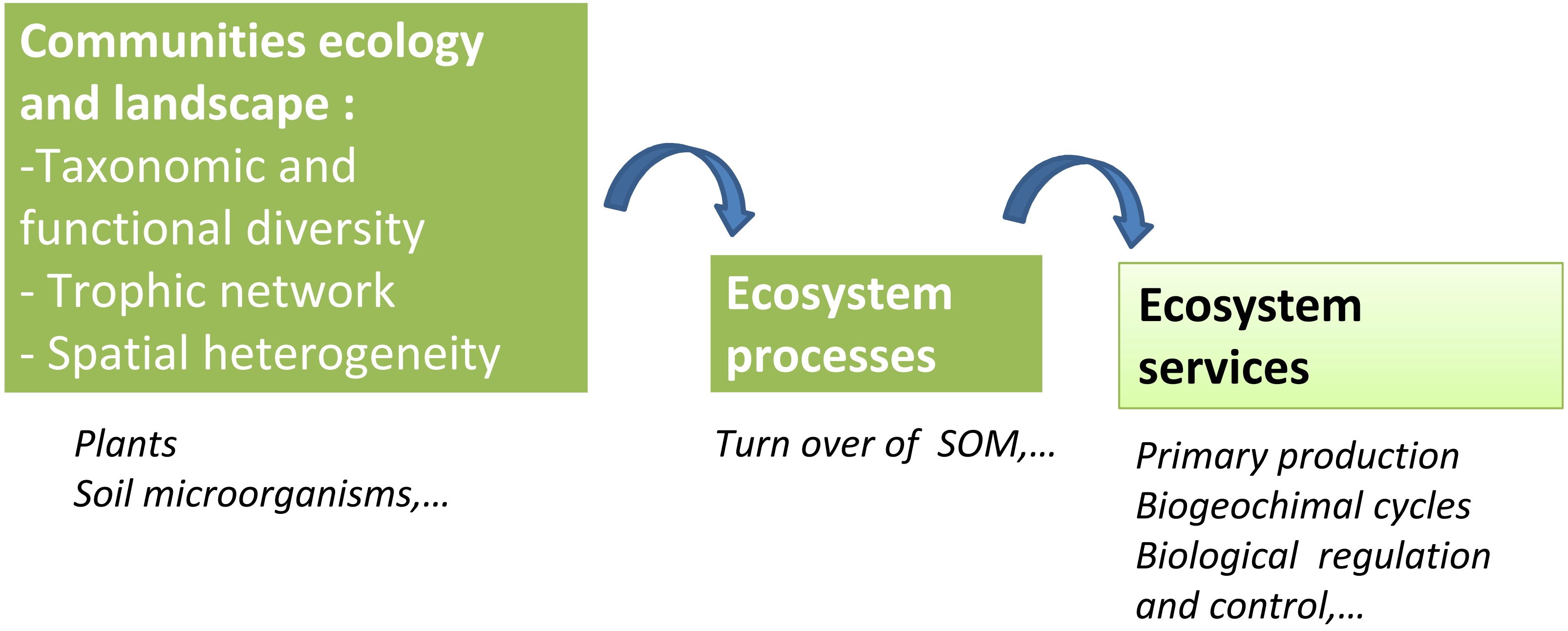






Agroecology

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



Communities ecology and landscape :
-Taxonomic and functional diversity
- Trophic network
- Spatial heterogeneity

Plants
Soil microorganisms,…

Ecosystem processes

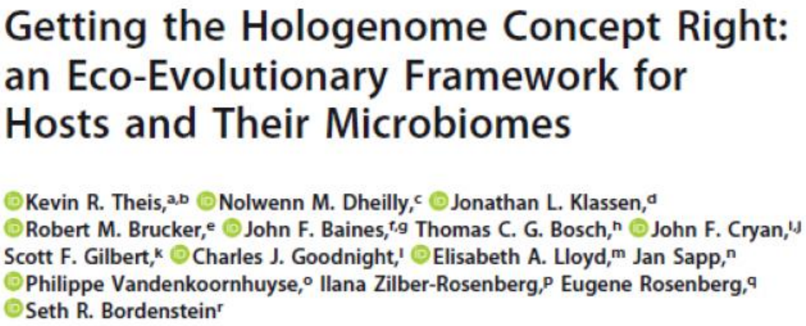
Turn over of SOM,…

Ecosystem services

Primary production
Biogeochimal cycles
Biological regulation and control,…

  - Link the microbial biodiversity and the plant ecosystem

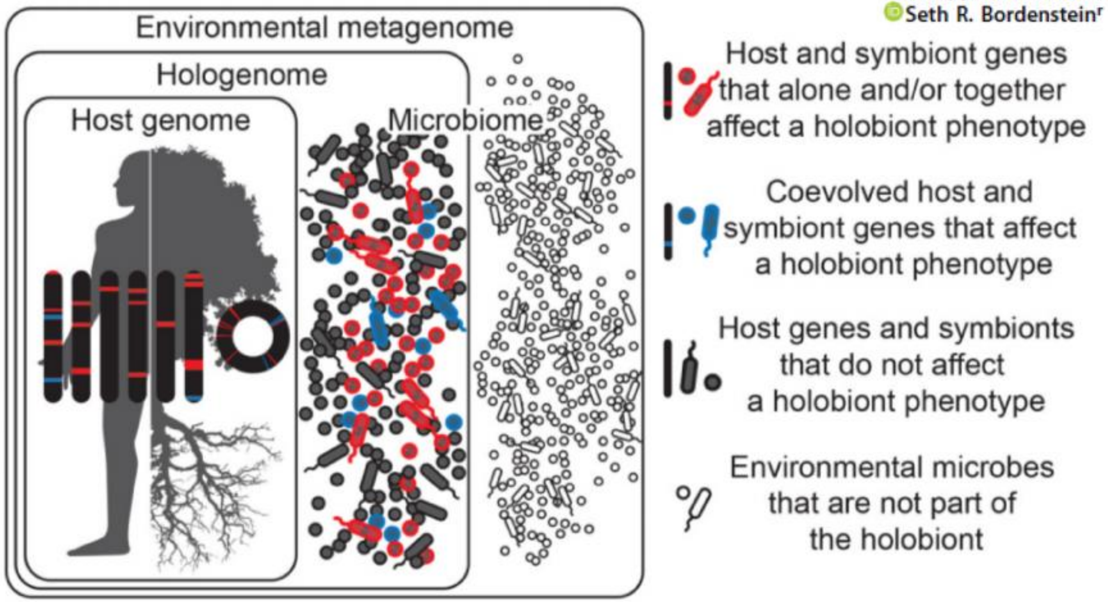
A conceptual framework: hologenome



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

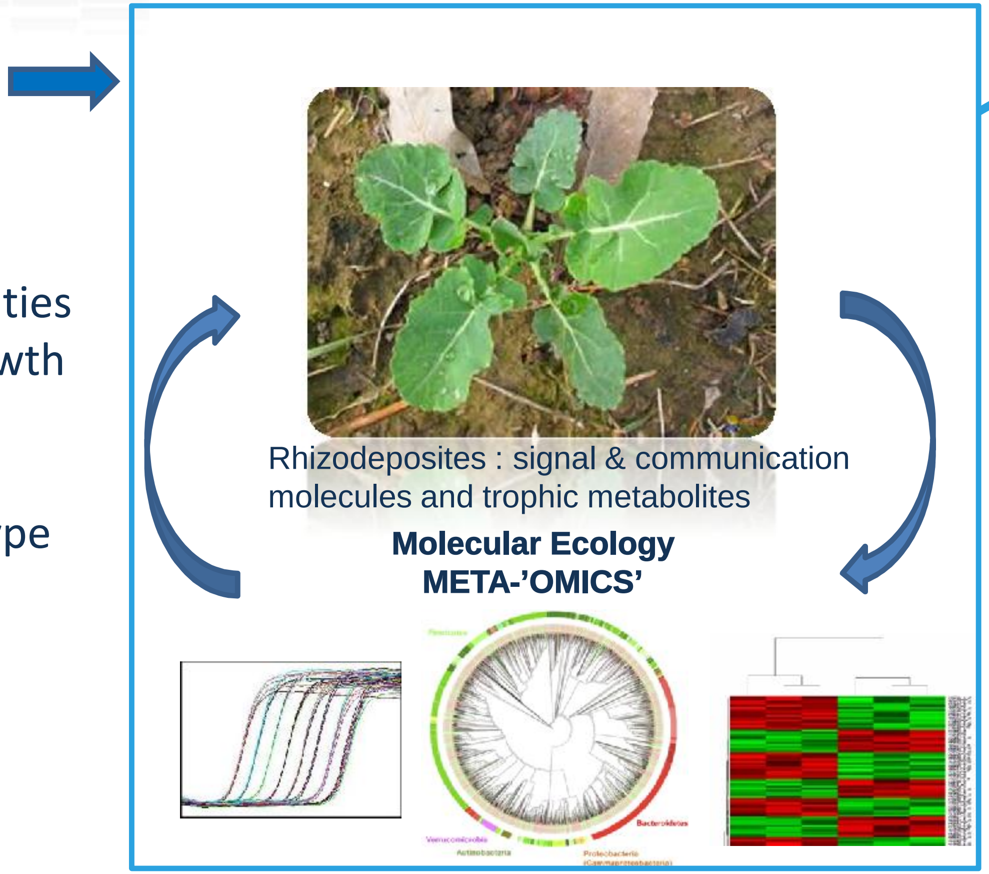
Kevin R. Theis,[a,b] Nolwenn M. Dheilly,[c] Jonathan L. Klassen,[d]
Robert M. Brucker,[e] John F. Baines,[f,g] Thomas C. G. Bosch,[h] John F. Cryan,[i,j]
Scott F. Gilbert,[k] Charles J. Goodnight,[l] Elisabeth A. Lloyd,[m] Jan Sapp,[n]
Philippe Vandenkoornhuyse,[o] Ilana Zilber-Rosenberg,[p] Eugene Rosenberg,[q]
Seth R. Bordenstein[r]

Theis et al. *mSystems* 2016



Moved from the plant phenotype = plant genotype x environment
to the plant extended phenotype = hologenome x environment

Feed back loop in plant - microbiote interactions



Light
CO_2
eg Environment factors

Hologenome =
Plant genotype x
Micobial
metagenome

Microbial communities effect on plant growth and health
=
Extended phenotype

Plant effect on microbial communities ecology
=
Rhizosphere effect

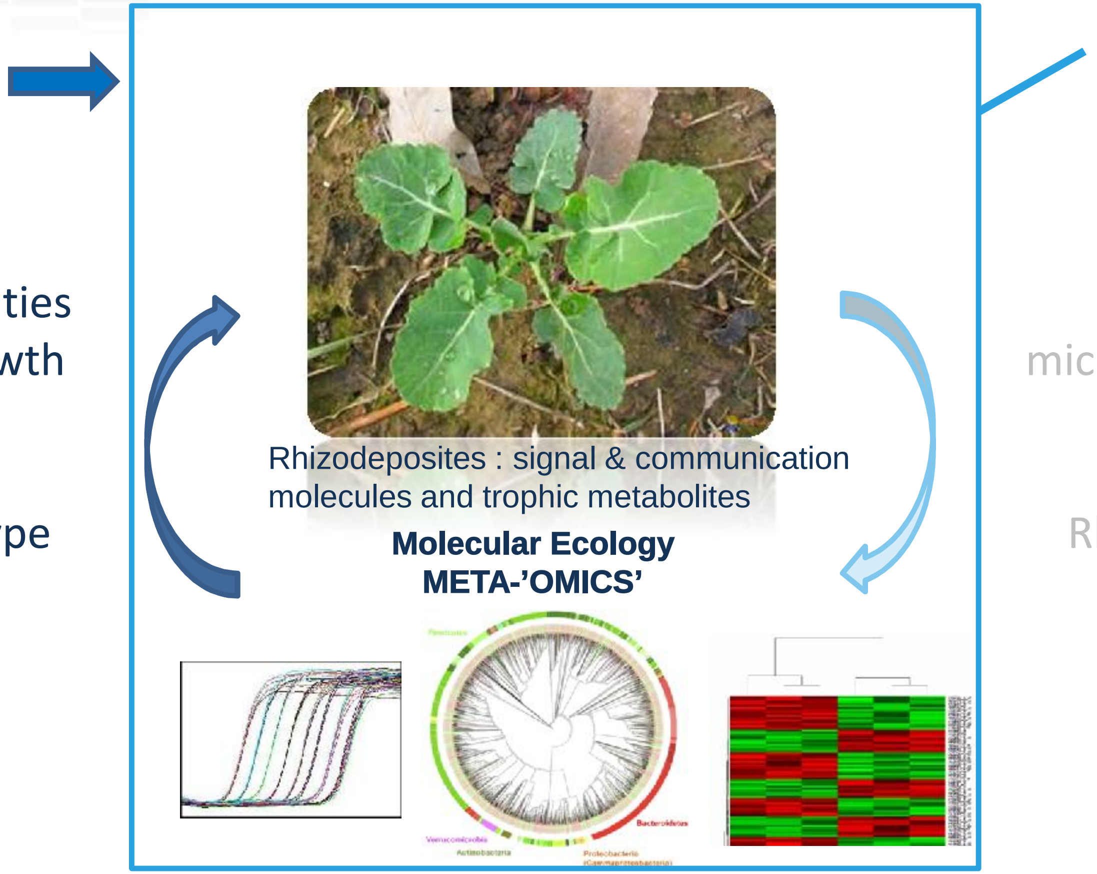
Rhizodeposites : signal & communication molecules and trophic metabolites

Molecular Ecology
META-'OMICS'

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

Feed back loop in plant - microbiote interactions

Light
CO_2
eg Environment factors



Rhizodeposites : signal & communication molecules and trophic metabolites

Molecular Ecology
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Hologenome =
Plant genotype x
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Microbial communities
effect on plant growth
and health
=
Extended phenotype

Plant effect on
microbial communities
ecology
=
Rhizosphere effect

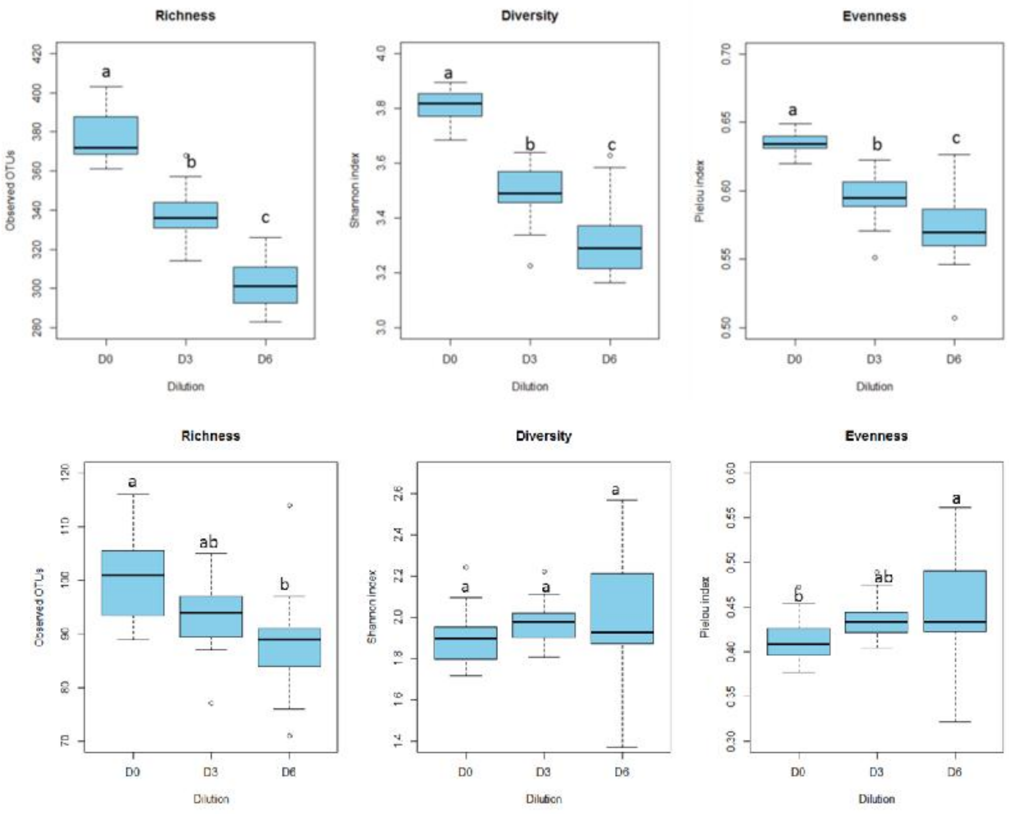
Microbial diversity manipulation : experimental diversity reduction

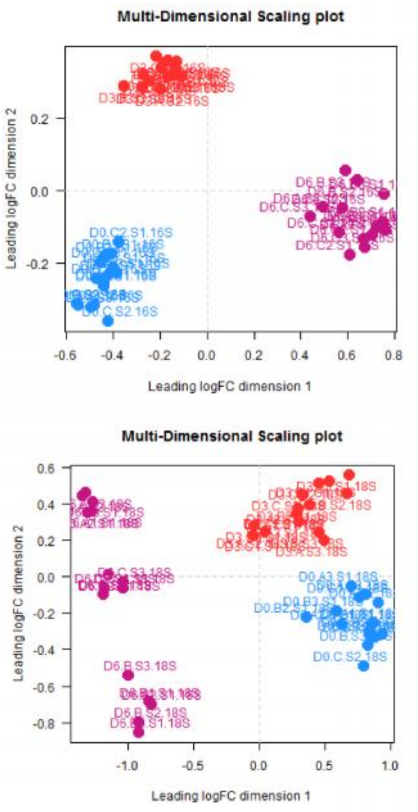
α diversity

β diversity

Bacteria

Fungi



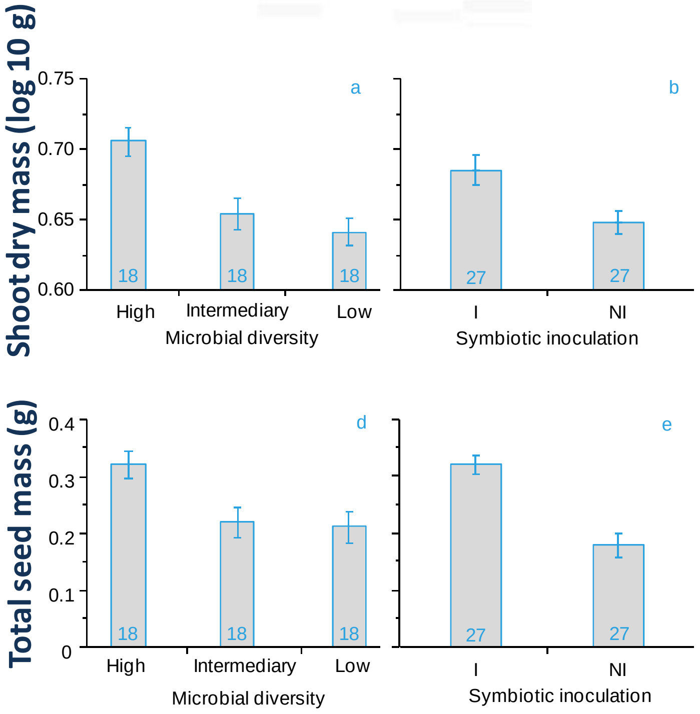


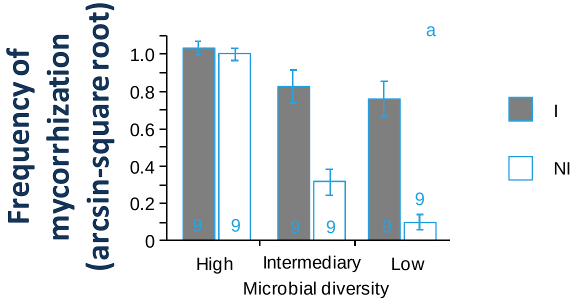
Richness (OTU number), Diversity (Shannon index) and Eveness (Piélou index)

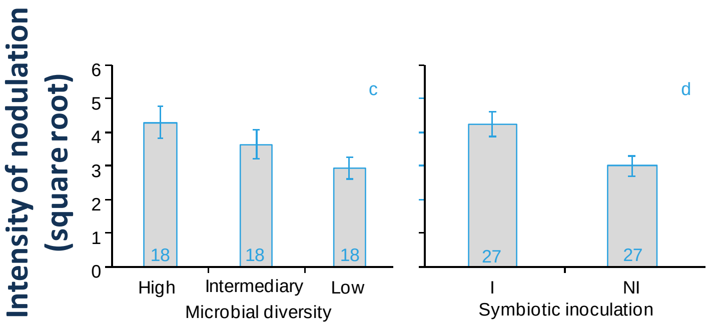
MDS on distance matrix (Bray and Curtis distance)

(Axis 1= 34% and axis 2 = 11%)

Effect of microbial communities diversity on *M. truncatula* fitness







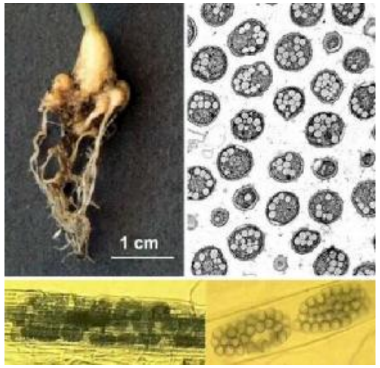
Lepinay, Mougel (XXXX) submitted

- ➢ **high soil microbial communities diversity is related to plant fitness and symbiosis processes**
- ➢ **how to manage it ?**

Holobiont – bioagressor interactions : a proof of concept

Oilseed rape holobionts

Plasmodiophora brassicae



PROTIST

Heterodera schachtii

NEMATODE

Delia radicum

INSECT

Leptosphaeria maculans

FUNGI

Brevicoryne brassicae

INSECT

Below ground **Above/below ground** **Above ground**

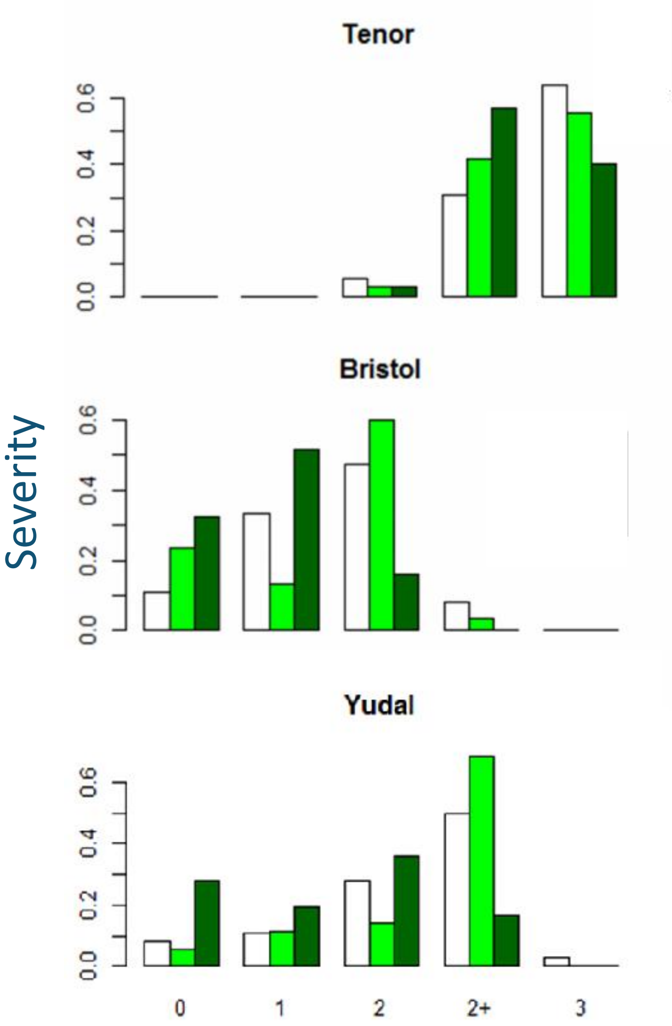
Plasmodiophora brassicaceae

Microbial diversity:

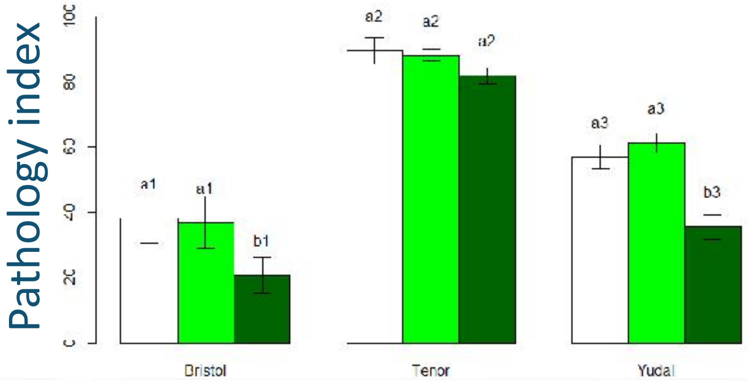
☐ D0 High

🟩 D3 Intermediary

■ D6 Low

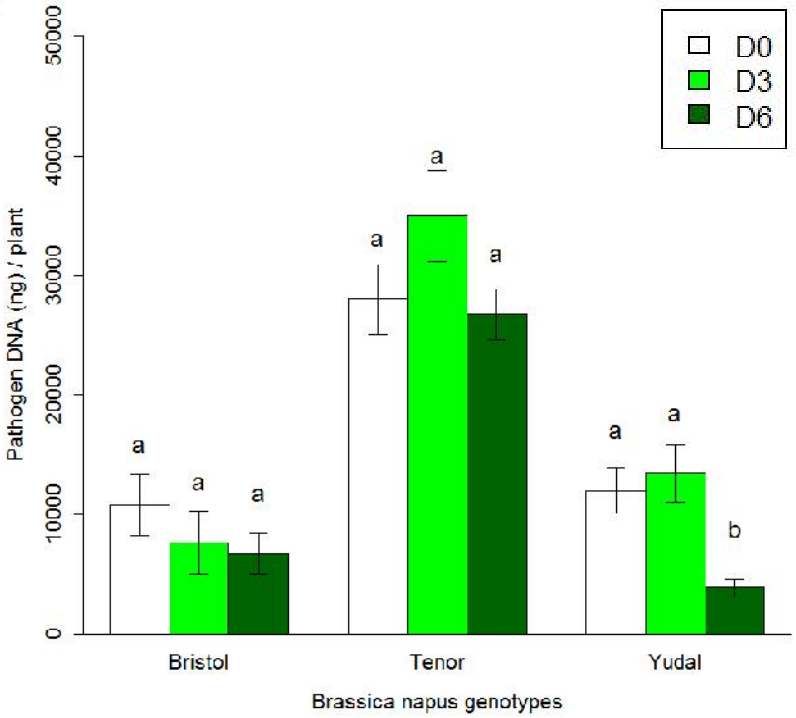


- 3 plant genotypes
  - Ténor = sensible
  - Yudal = tolerant
  - Bristol = resistant
- n=36 per dilution
- eH 10^7 spores/plant



➢ **pathology index modulated in relation with plant genotype and microbial communities diversity**

Plasmodiophora brassicaceae



Microbial diversity:
High
Intermediary
Low

- ➢ **the pathogen fitness (DNA amount) is affected by microbial diversity, effect on the tolerant plant genotype**
- ➢ **perspective : functional analysis thanks to metatranscriptomics analysis of the holobiont in relation with pathogen (genomic data available)**

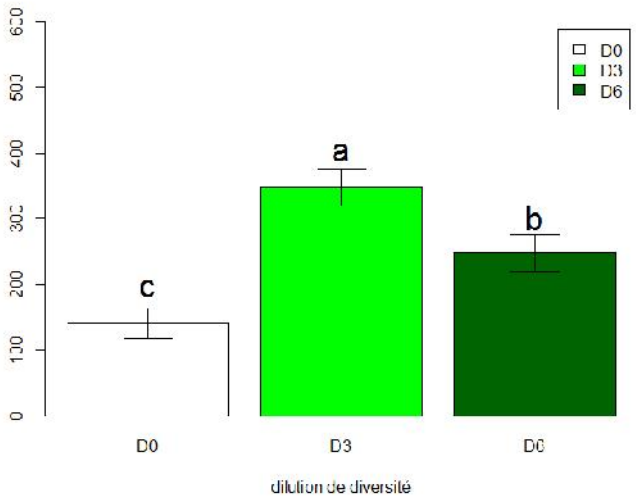
Heterodera schachtii

Microbial diversity:

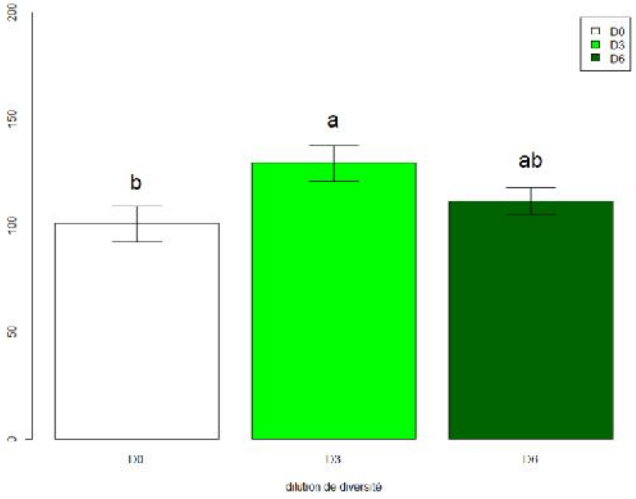
☐ D0 High
🟩 D3 Intermediary
■ D6 Low

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

Number of cysts formed



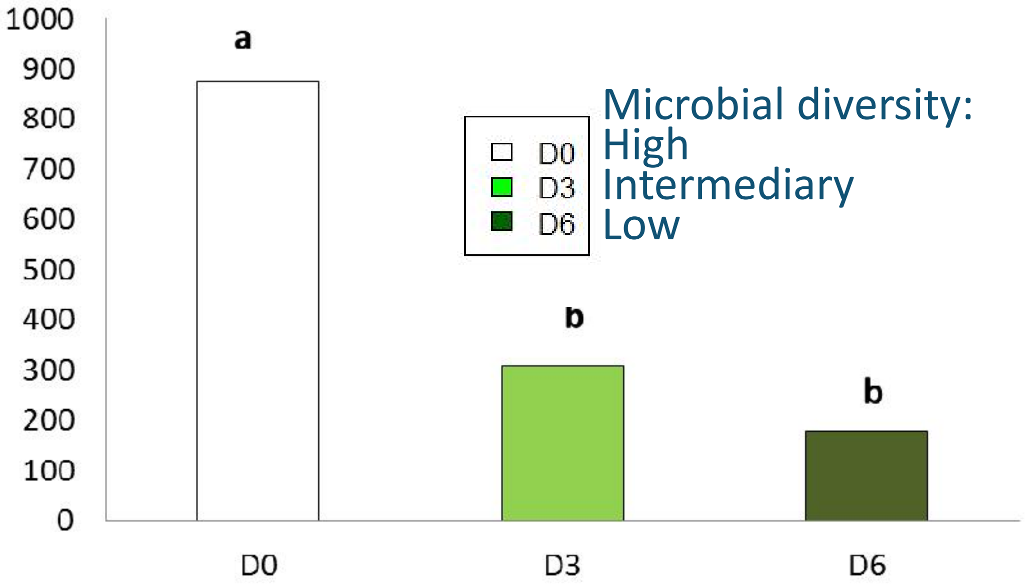
Number of larvae / cyst (n=10 cysts/plant)



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

Heterodera schachtii

Number of larvae in parental cyst (n=8 cysts/plant)



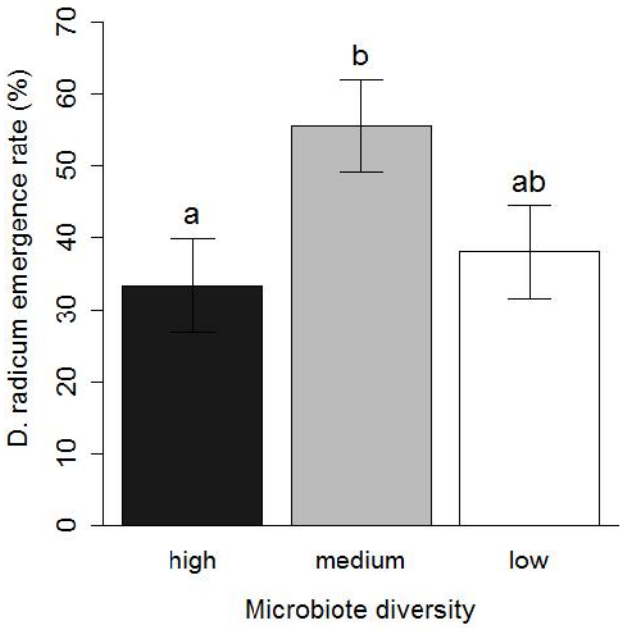
Microbial diversity:
High
Intermediary
Low

- ➢ **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)**

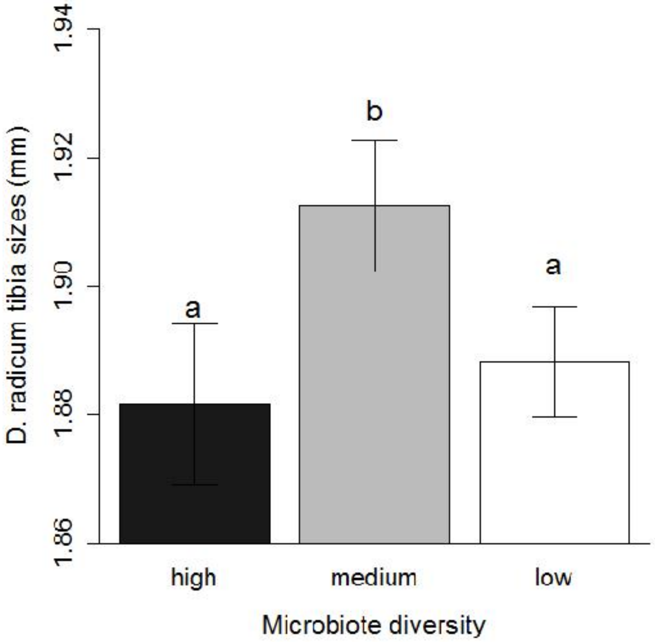
Delia radicum

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

Emergence rate



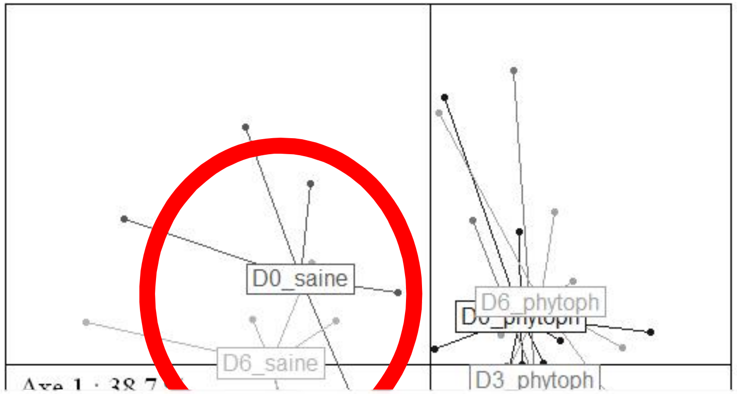
Tibia length

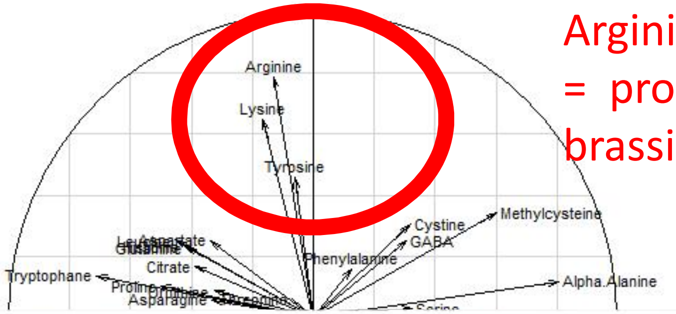


(Statistic test= Wilcoxon & Kruskal-Wallis)

➢ **Emergence rate and the fitness of the root fly emergent were affected by microbial diversity**

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





Amino acid

Arginine
= proteins involved in brassica **defense**

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

st

(Statistic test= Wilcoxon & Kruskal-Wallis)

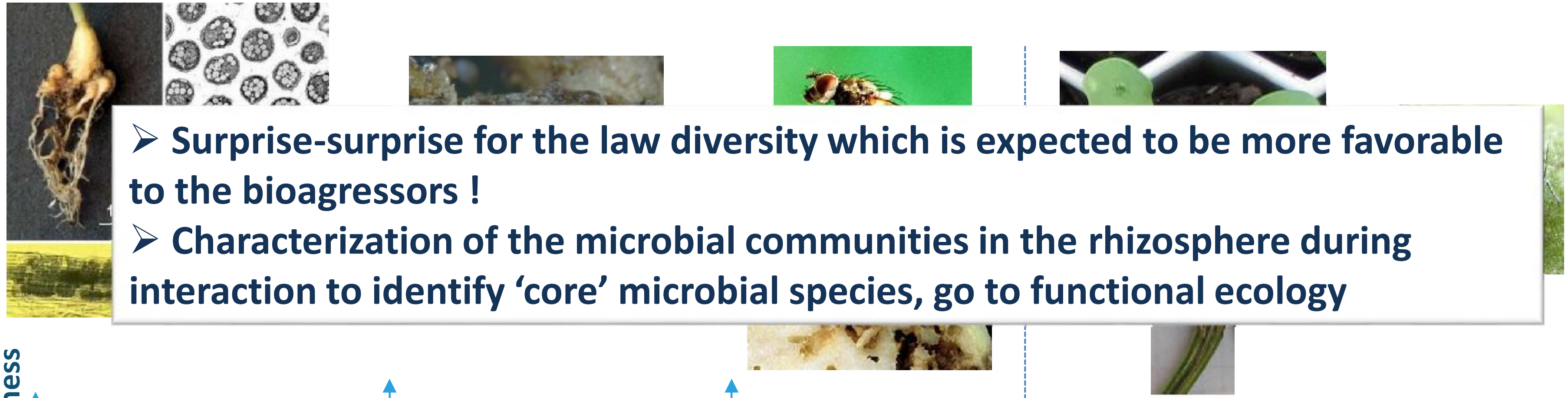
(Test statistique = PLS-DA)

(Lachaise, Cortesero, Mougel, unpublished)

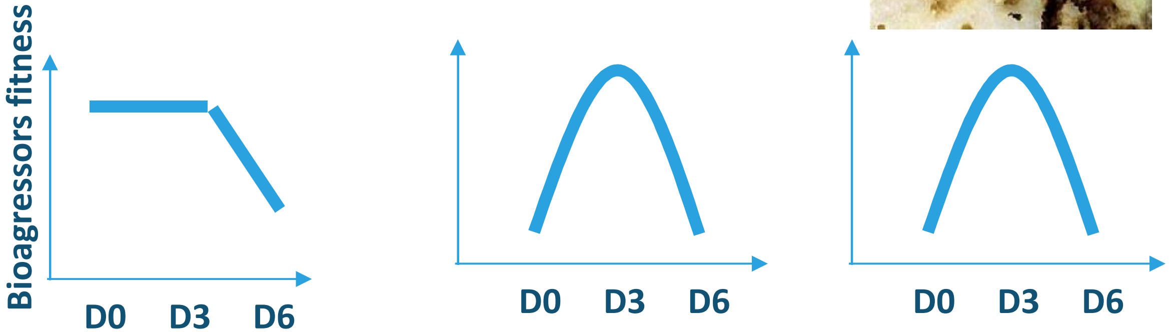
Summary

Oilseed rape holobionts

Plasmodiophora brassicae | *Heterodera schachtii* | *Delia radium* | *Leptosphaeria maculans* | *Brevicoryne brassicae*



- ➢ Surprise-surprise for the law diversity which is expected to be more favorable to the bioagressors !
- ➢ Characterization of the microbial communities in the rhizosphere during interaction to identify ‘core’ microbial species, go to functional ecology



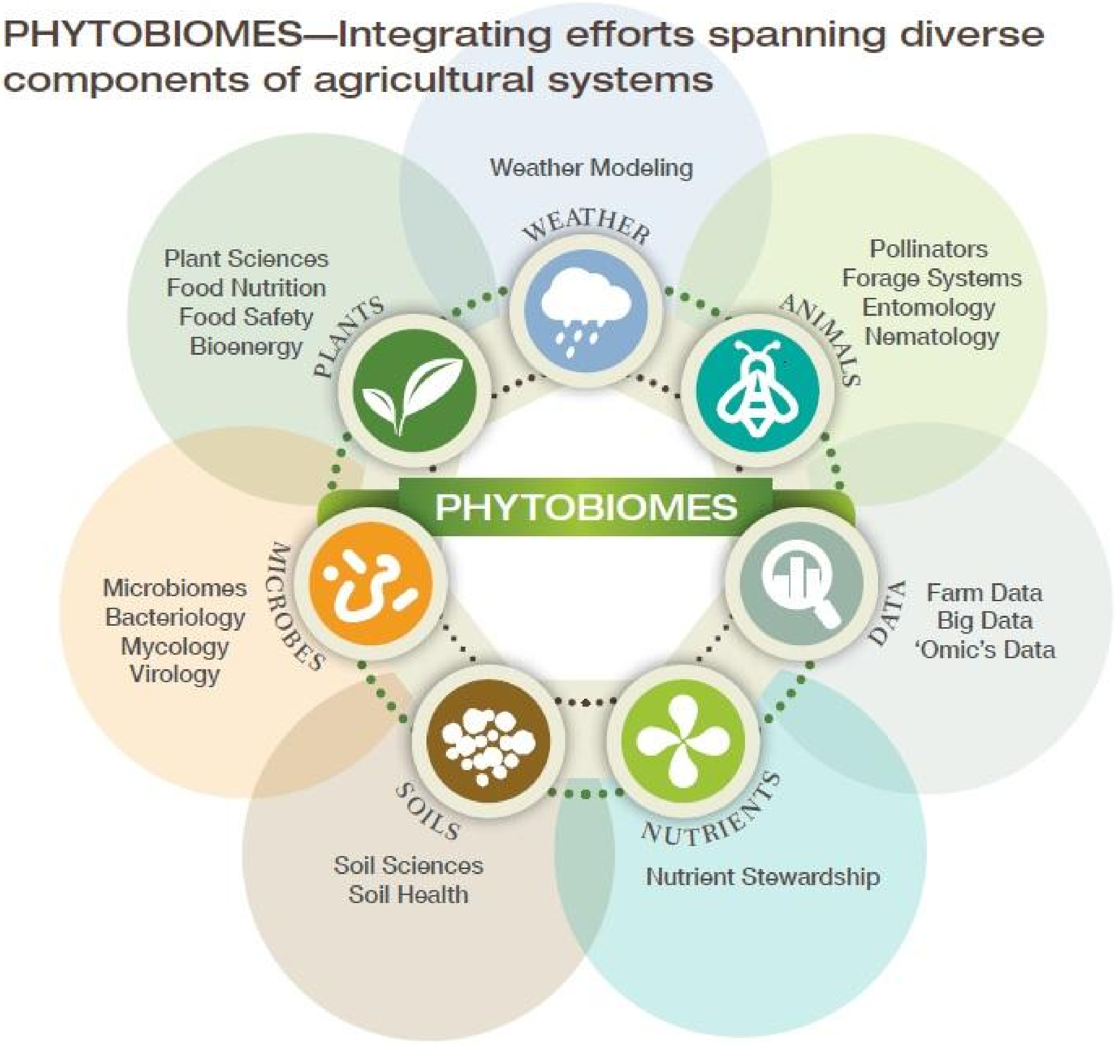
Below ground

NS **NS**

Above ground

(Mougel *et al*., en préparation)

Phytobiomes US Initiative & Alliance


PHYTOBIOMES—Integrating efforts spanning diverse components of agricultural systems

100 miliions US $ / year
(10 years founding)
but that was before …

A collaborative work !

Thanks for your attention !

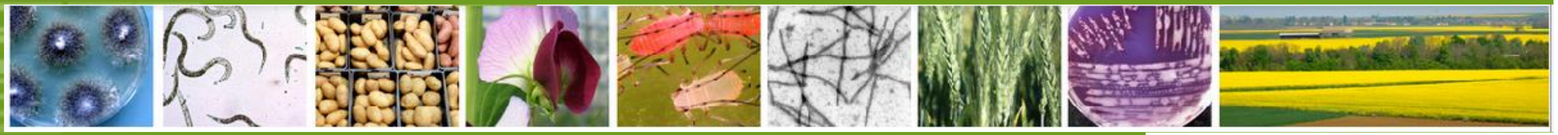
Plant-microbial communities interactions : influence on plant health and growth

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

Institute for Genetics, Environment and Plant Protection - IGEPP



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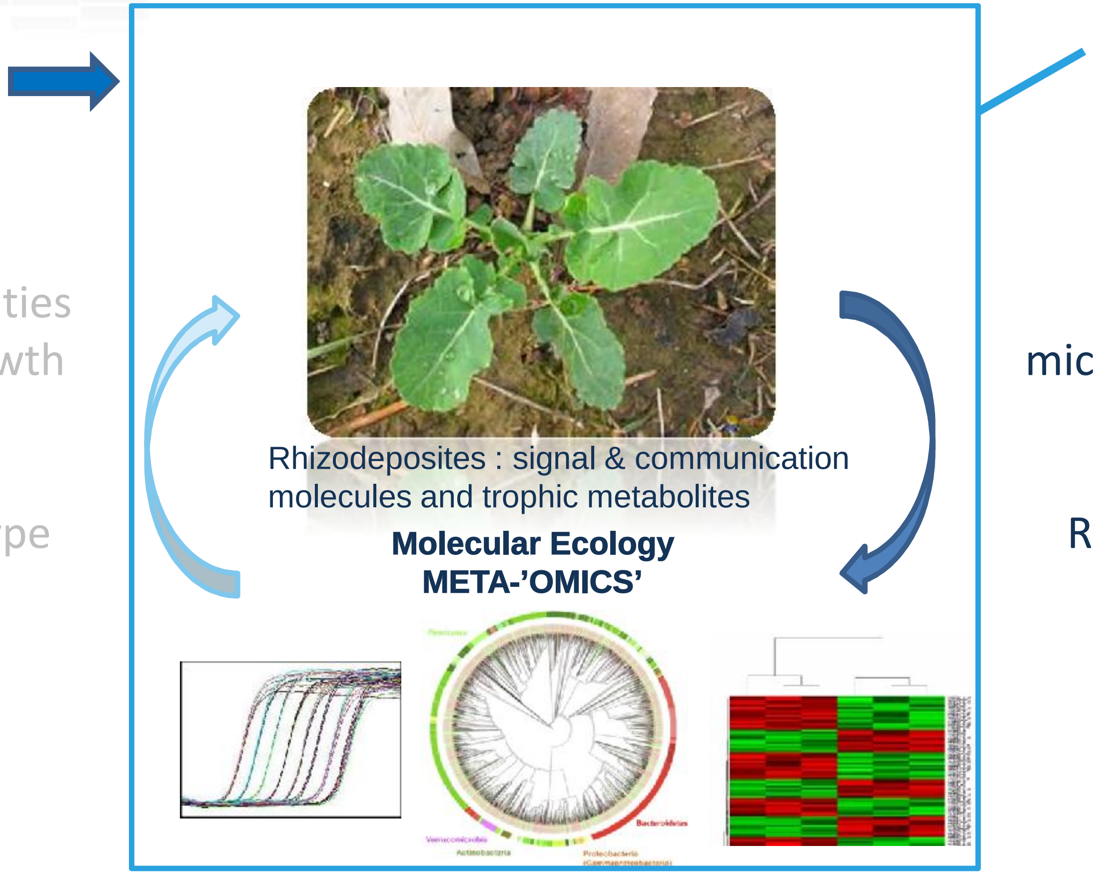
Feed back loop in plant - microbiote interactions



Light
CO_2
eg Environment factors

Microbial communities
effect on plant growth
and health
=
Extended phenotype

Rhizodeposites : signal & communication molecules and trophic metabolites

Molecular Ecology
META-'OMICS'

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 rhizophere) < endophytes**
- ➢ **Dynamic effect depending of plant phenology (vegetative versus reproducive stages)**
- ➢ **Core-microbiote with quantitative variations**

Mougel et la., New Phytologist, 2006
Lundberg et al., Nature, 2012
Bulgarelli et al., Nature, 2012
Schlaeppi et al., PNAS, 2013

PNAS

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

Klaus Schlaeppi[a,b], Nina Dombrowski[a], Ruben Garrido Oter[a,c,d], Emiel Ver Loren van Themaat[a], and Paul Schulze-Lefert[a,d,1]

[a]Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany; [b]Plant–Soil-Interactions, Institute for Sustainability Sciences, Agroscope, Reckenholzstrasse 191, 8046 Zurich, Switzerland; [c]Department of Algorithmic Bioinformatics, Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany; and [d]Cluster of Excellence on Plant Sciences (CEPLAS), Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

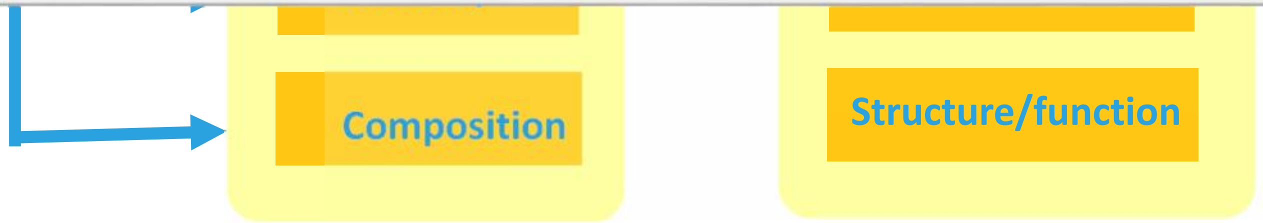
A genome wide analysis study GWAS to identify keys plant genes

Host genotype

192 accessions of M. truncatula (core collection)

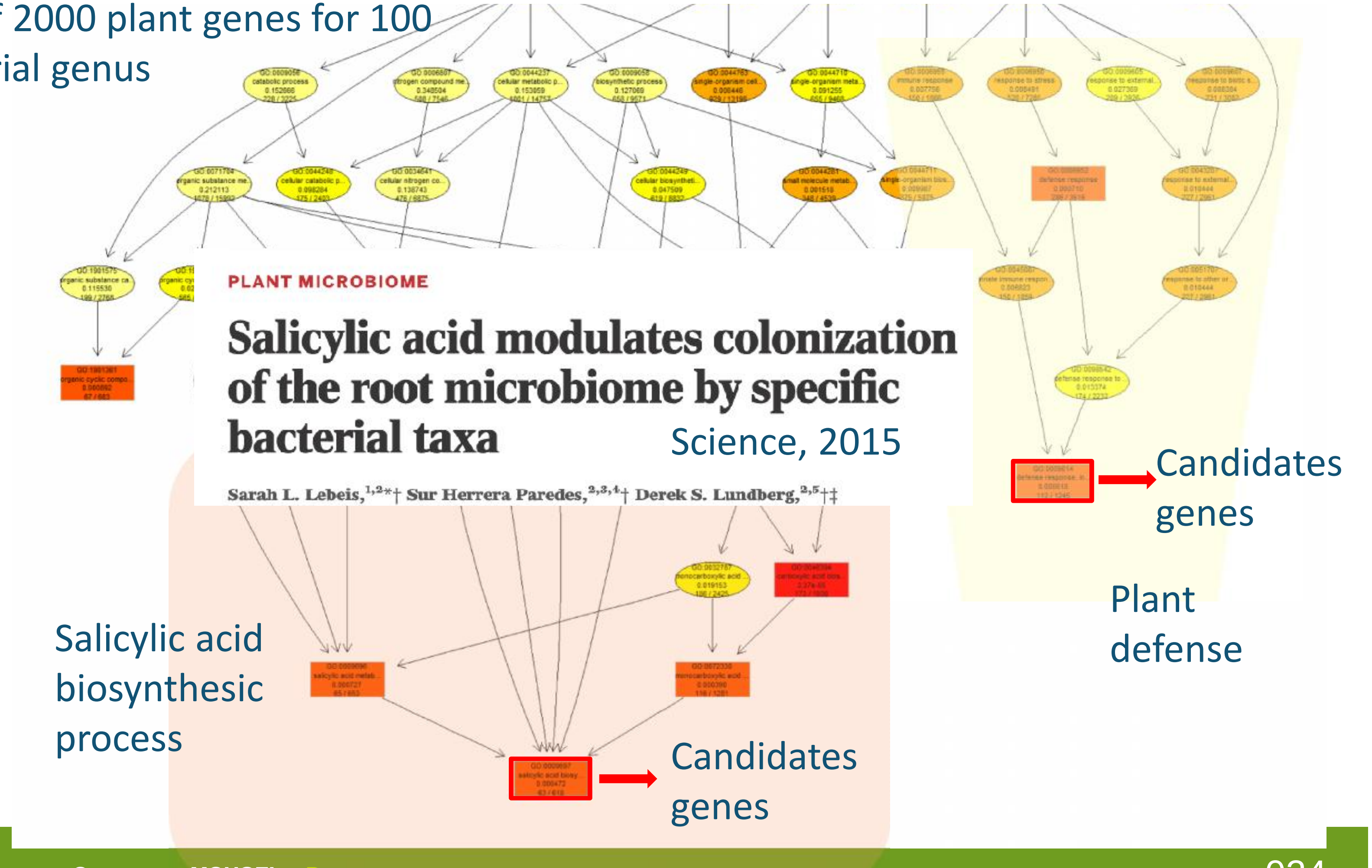
- 191 on the 876 bacterial species share a significant heritability values (between 0,2 and 0,6)
- Genetic analysis allow the identification of plant genes in relation with signalisation, N metabolism and stimuli response (stress et plant defense)
- Relevant genes for plant breeders ?

Zancarini, Mougel, in preparation



Global analysis of significant plant genes related to dominant microbial 'species'

✓Singular Enrichment Analysis (Classic Ficher test, TopGO R) : list of 2000 plant genes for 100 dominant bacterial genus



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†‡]

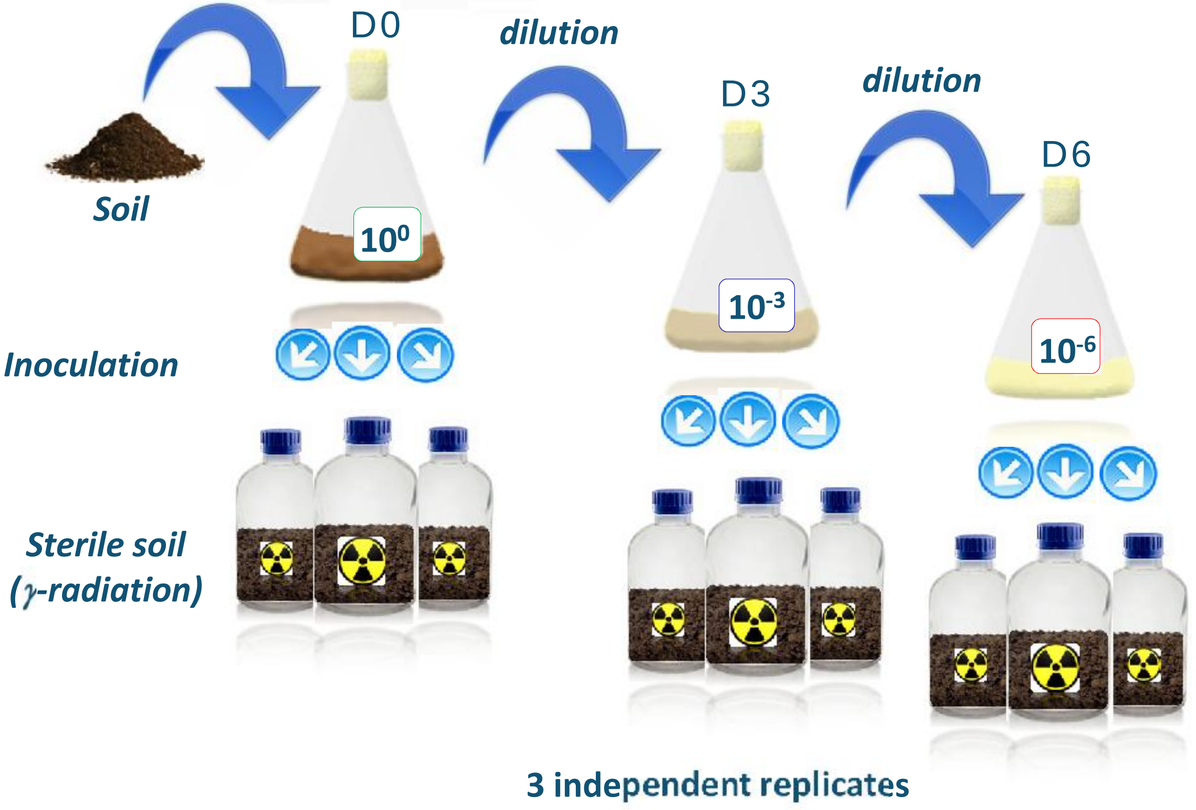
Candidates genes

Plant defense

Salicylic acid biosynthesic process

Candidates genes

Microbial diversity manipulation : experimental diversity reduction



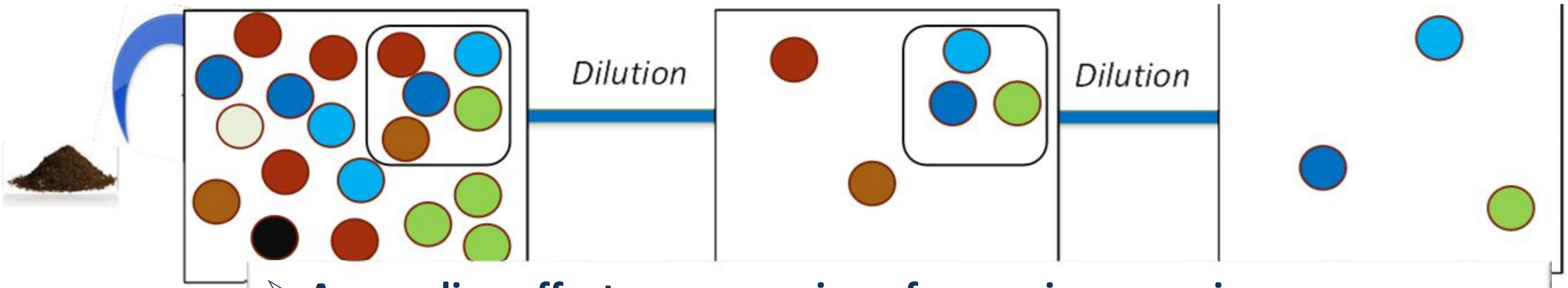
- Abundance (Q-PCR)
- Diversity (MiSeq)
- Soil physico-chemical properties

6 weeks pre-incubation

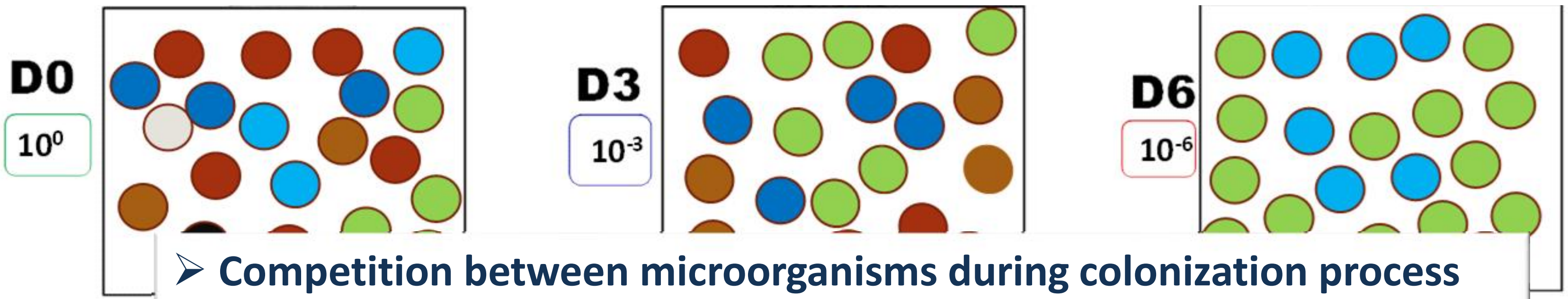
Sowing

- Plant biomass and metabolites
- Life history traits of bioagressors
- Microbial diversity

Reduction of rare soil microbes and new microbial communities assemblages



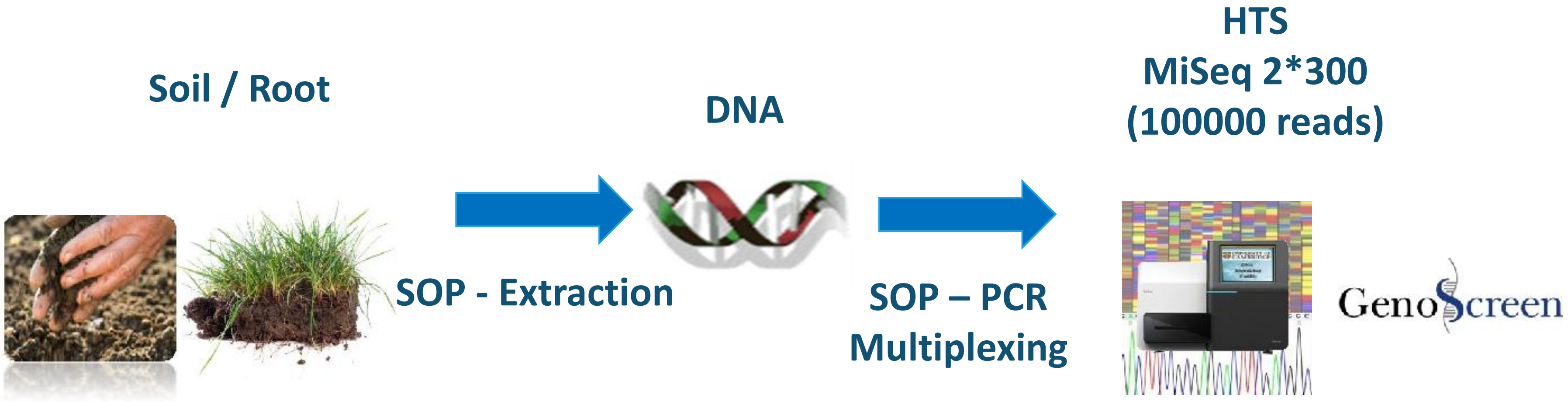
- A sampling effect = suppression of rare microorganisms



- Competition between microorganisms during colonization process (ie less diversity is supposed to select dominant species and high competitors for nutrient – R strategist, copiotroph)

Microbial diversity analysis (1/2)

- Biology



Soil / Root

SOP - Extraction

DNA

SOP – PCR
Multiplexing

HTS
MiSeq 2*300
(100000 reads)

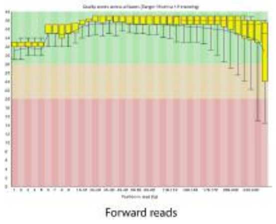
GenoScreen

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



Denoising
Trimming

Clean reads

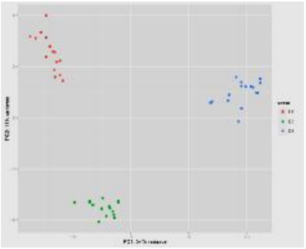
Workflow GnS-PIPE

Derplication
Clustering
Alignment
Taxonomy

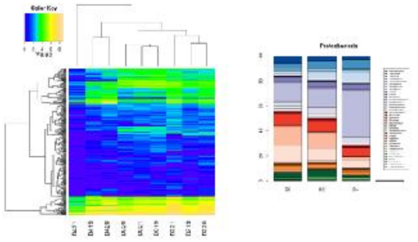
Count table
Diversity index

- Statistics (coll J. Aubert)

Classification



Discriminant analysis

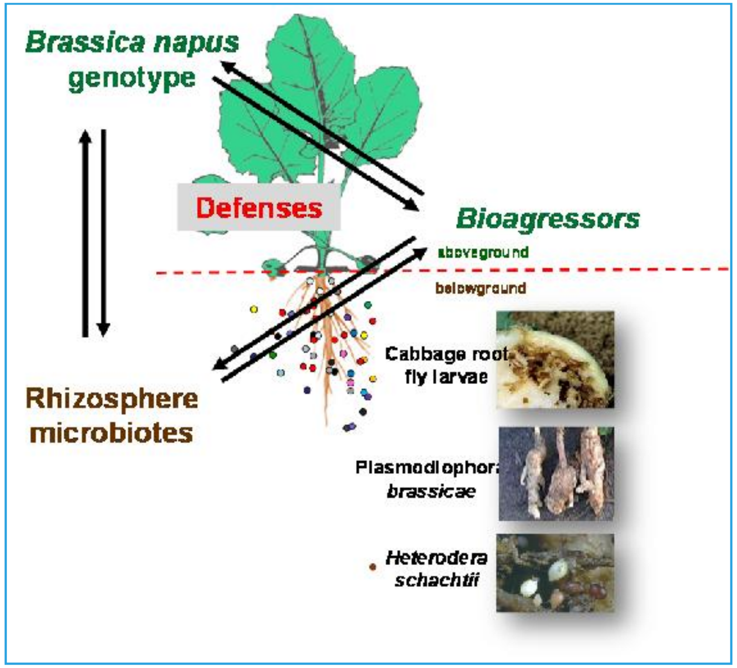


Take home message

- The nature of holobiont (micobial diversity associated with plant) modulate plant metabolites => direct or indirect effect on bioagressor (signalisation, trophic, defense)

- The plant holobiont influence bioagressor behaviours but not in a linear relationship => identification of key microbial players (direct antagonism ?)

- Coupling diversity and functional analysis are in perspectives to understand the extended phenotype



- A more holistic and integrated issues to improve plant adaptation in agrosystem