



Génétique  
Quantitative  
et Évolution  
Le Moulon



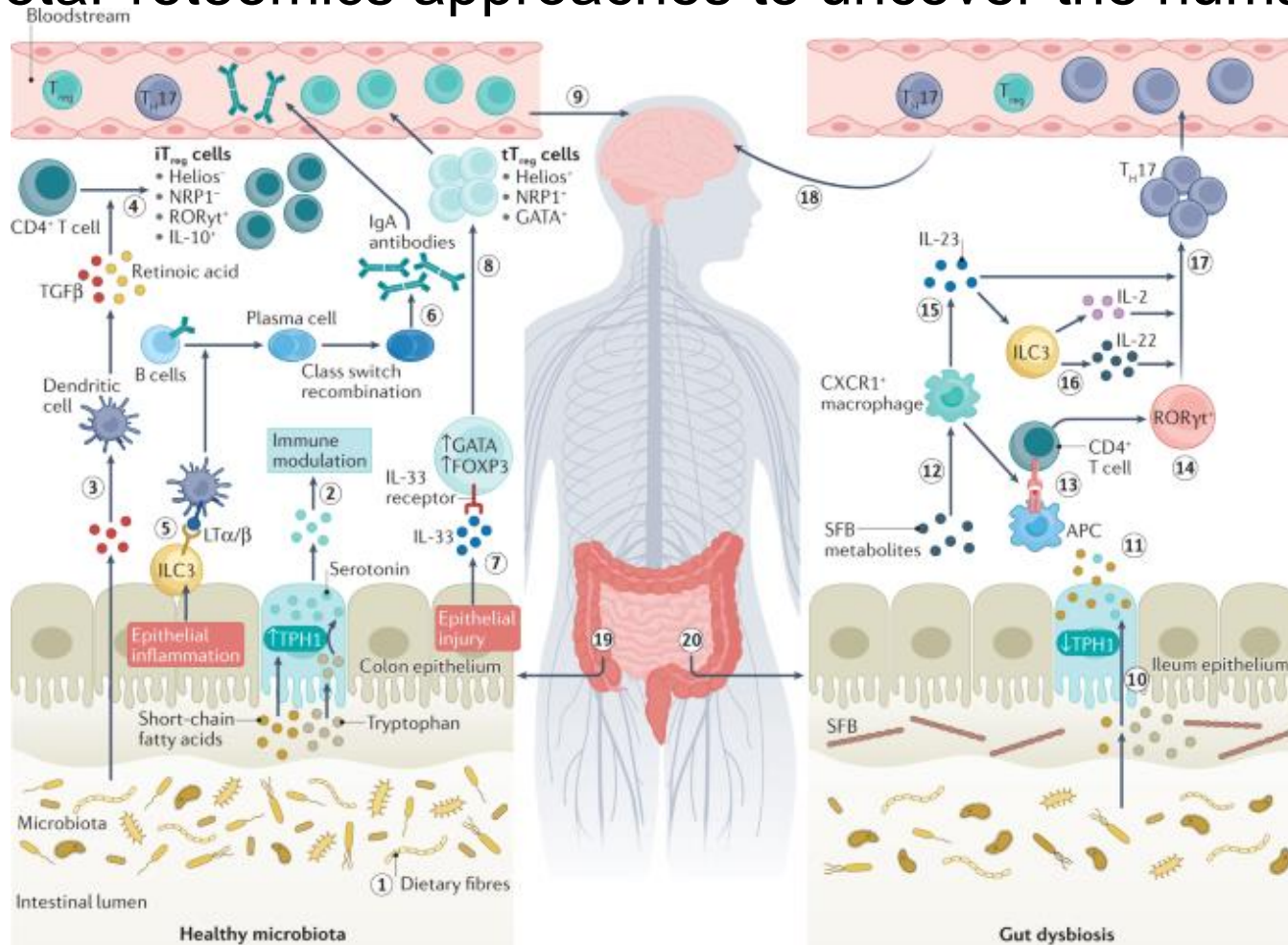
# MetaP-Rhiz:

## A project to better understand the rhizosphere microbiome

Willy Bienvenut, GQE – Le Moulon, IDEEV, Gif/Yvette, France

# Meta-What ... ?

MetaProteomics approaches to uncover the human microbiome...



“Gut microbiota is intricately linked to human health and disease...”

“Modulation of the microbiome could be therapeutically beneficial...”

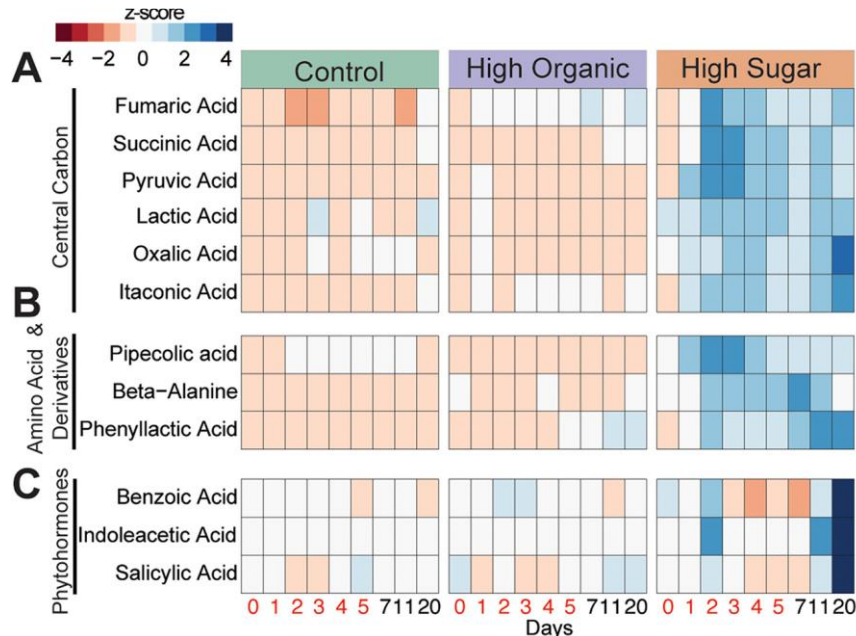
The role of the gut microbiota in multiple sclerosis.

Correale *et al. Nat. Rev. Neurol. Subsets*, 2022, DOI:10.1038/s41582-022-00697-8

# Rhizosphere... ?

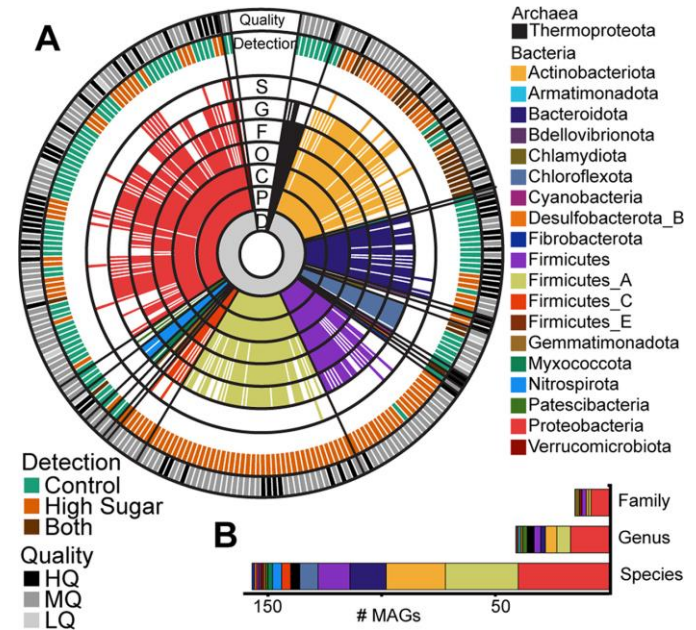
Variation in Root Exudate Composition Influences Soil Microbiome Membership and Function.

Seitz et al., *App. Environ. Microbiol.* **2022**, DOI: 10.1128/aem.00226-22



Exometabolites abundances in root exudate across 3 different treatments on sorghum

“Soil microbial community [...] is influenced by root exudates...”



Taxonomy of the 243 dereplicated metagenome-assembled genomes

“Changes in [...] microbiome result in altered production of plant-relevant metabolites...”

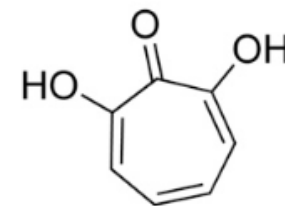
“Multiomics [...] analyses of [...] soil microbiomes provide new information on plant-soil environments useful for the development of efficient and precise microbiote management strategies in agricultural systems.

# Plant-Bacteria Interactions

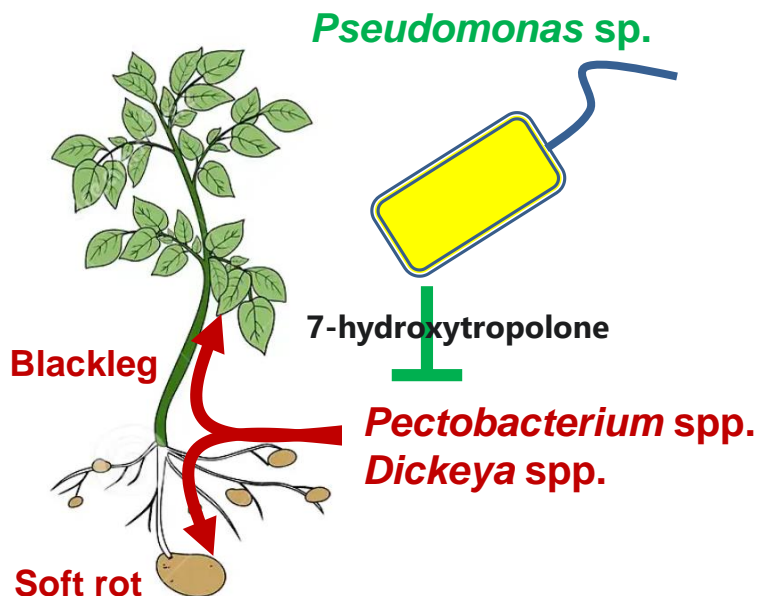


Group Leaders

D. FAURE & P. MERGAERT



Development of **biocontrol tools** for **protecting potato crops** against *Pectobacterium* and *Dickeya*



## Challenge:

To fight against *Dickeya* and *Pectobacterium* pathogens

## Research work:

Biocontrol agent formulation and field trials

## Deliverable:

Optimized formulation and its use in potato fields



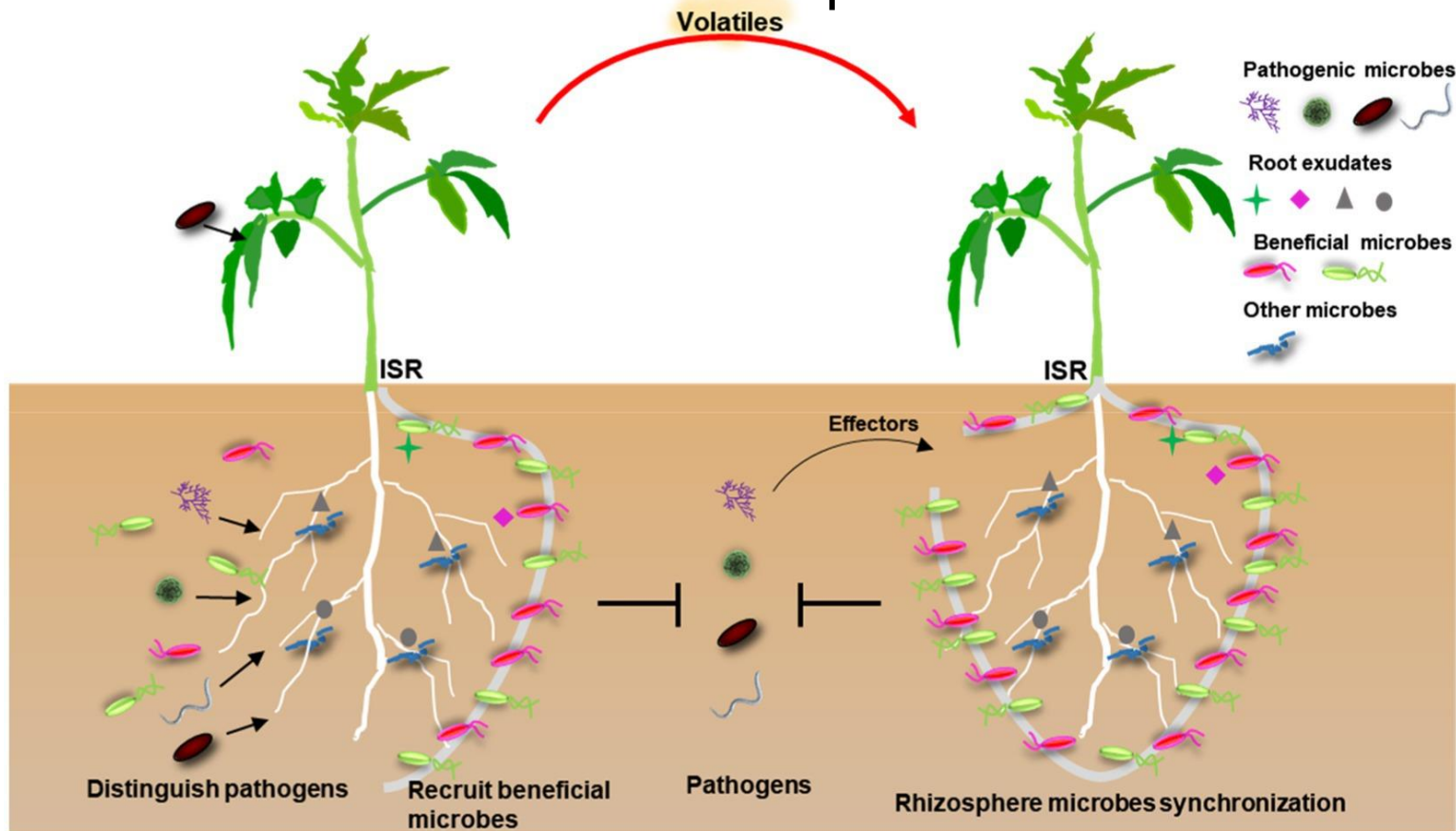
SaveTuber  
2024-2026

EcoPhytoMaturation2023



# MetaP-Rhiz... ?

MetaProteomics approaches to uncover the functionalities associated to the rhizosphere microbiota



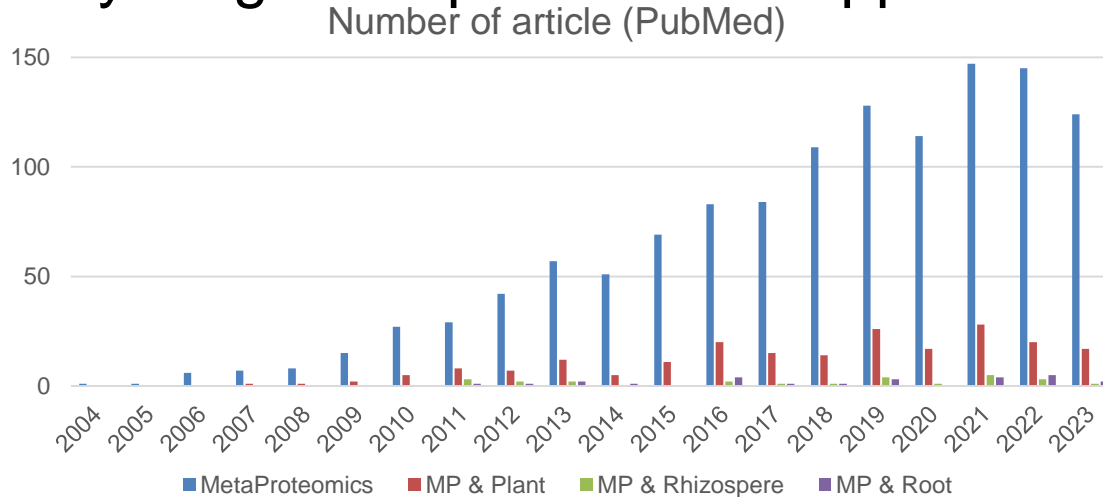
A conceptual model of pathogen-mediated rhizosphere microbial recruitment for plant protection.

Rhizosphere Microbiome: The Emerging Barrier in Plant-Pathogen Interactions

Li et al. *Front. Microbiol.*, 2021, DOI: 10.3389/fmicb.2021.772420

# Rhizosphere metaProteomics

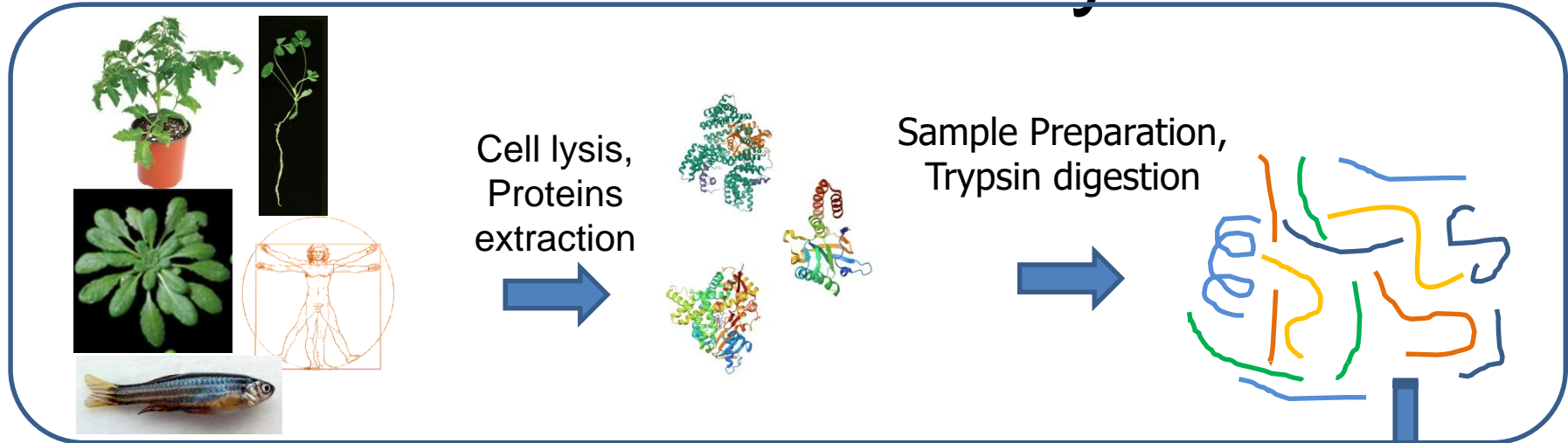
- The early stages of "plant metaP approaches "



- Two types of investigations:

- metaP in wild soil:
  - Effect of climate change in Arctic lands / permafrost...
  - Influence of flood in rivers plains,
  - Evolution of polluted soils...
- metaP in agronomy:
  - Plants and soil microbiomes interaction knowledge
  - Maintain and increase food production
  - Decrease of chemical inputs...

# Proteomics analyses



## Proteins sequence DB

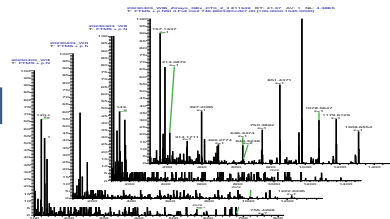
Generic: NCBIInr, UniProtKB, SwissProt...  
Genomic: Whole Genome Sequencing...  
mRNA sequencing  
metaGenomics...

Quantitation,  
Associated functions,  
Cellular pathways

X!Tandem



Protein  
Identification Tool



List of identified  
proteins



# Is that simple... ?



## Samples contain:

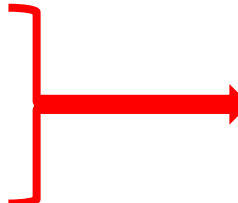
### Plant:

- Roots 
- Microbiote 

Not the major target

**Major target**


### Alternative organisms:

- Insects
  - Invertebrates
  - Vertebrate
  - ...
- 

**Contaminants!!!**

False positive identifications  
May trigger suppression effects...

### Organic material

- Rotten "plants" parts...
  - Pigments, tanins...
- 

**Impair protein extraction  
& LC-MS analyses**

- Nutrients 

Minor impairing effects

...



# Sample preparation

## 2 options:

### Indirect:

- microbiote extraction
- Extracted cells lysis
- Protein extraction



### Advantages

Better sample quality

- Low amount of contaminants
- Mostly related to the microbiome

### Drawbacks

Multiple steps extraction...

Low amount of material

**Very low number of identified proteins**

### Direct

- Cell lysis
- Protein extraction



### Advantages

Single step extraction

Good amount of material for LC-MS

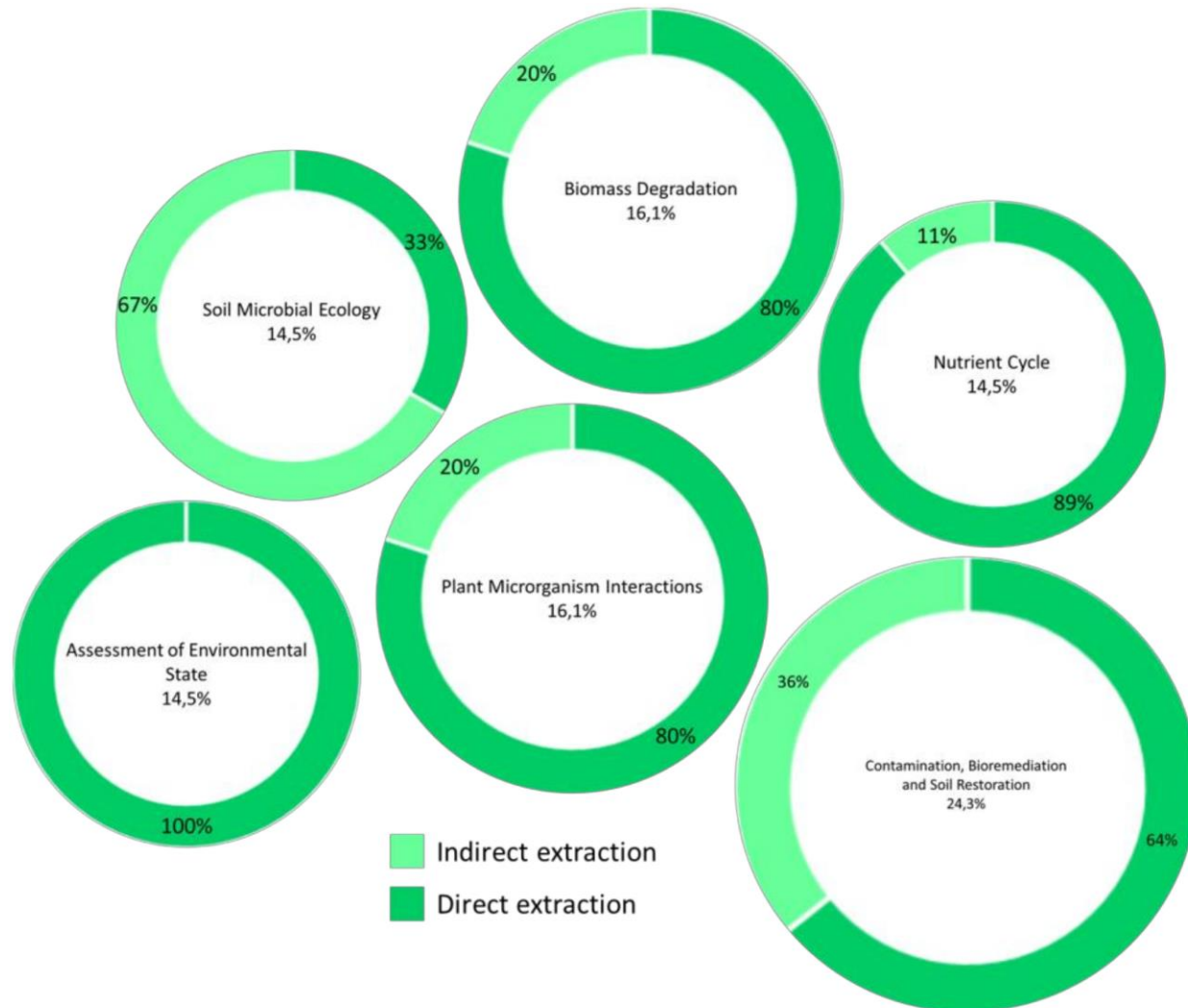
High number of identified proteins

### Drawbacks

Extracted material highly contaminated

- by tanins
- other species, even plants...

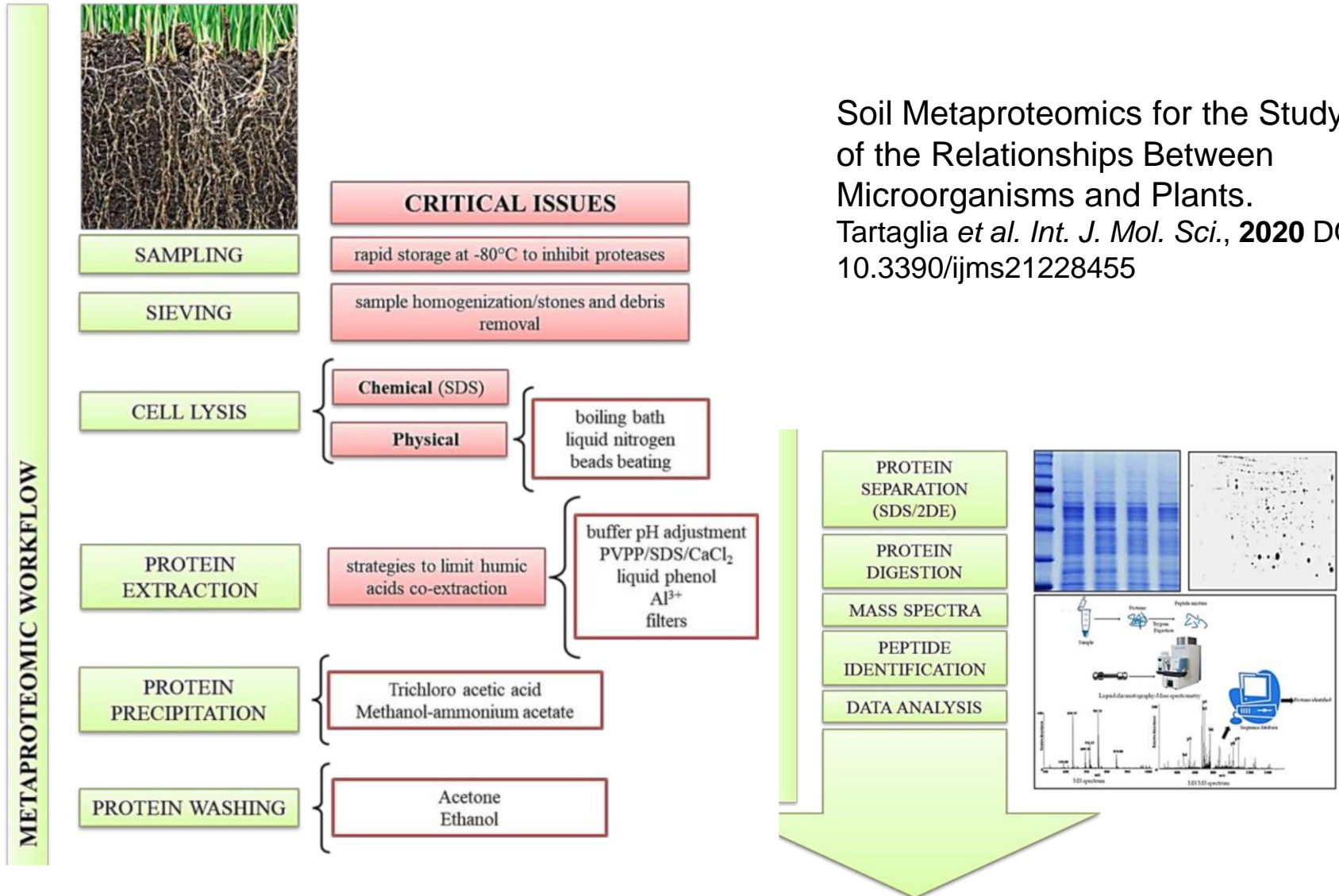
# Direct / Indirect...



Soil Metaproteomics for the Study of the Relationships Between Microorganisms and Plants:  
A Review of Extraction Protocols and Ecological Insights

Tartaglia *et al. Int. J. Mol. Sci.* **2020**, DOI: 10.3390/ijms21228455

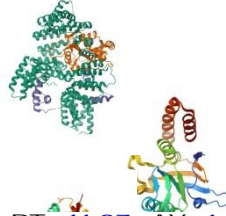
# One example



# Proteomics analyses



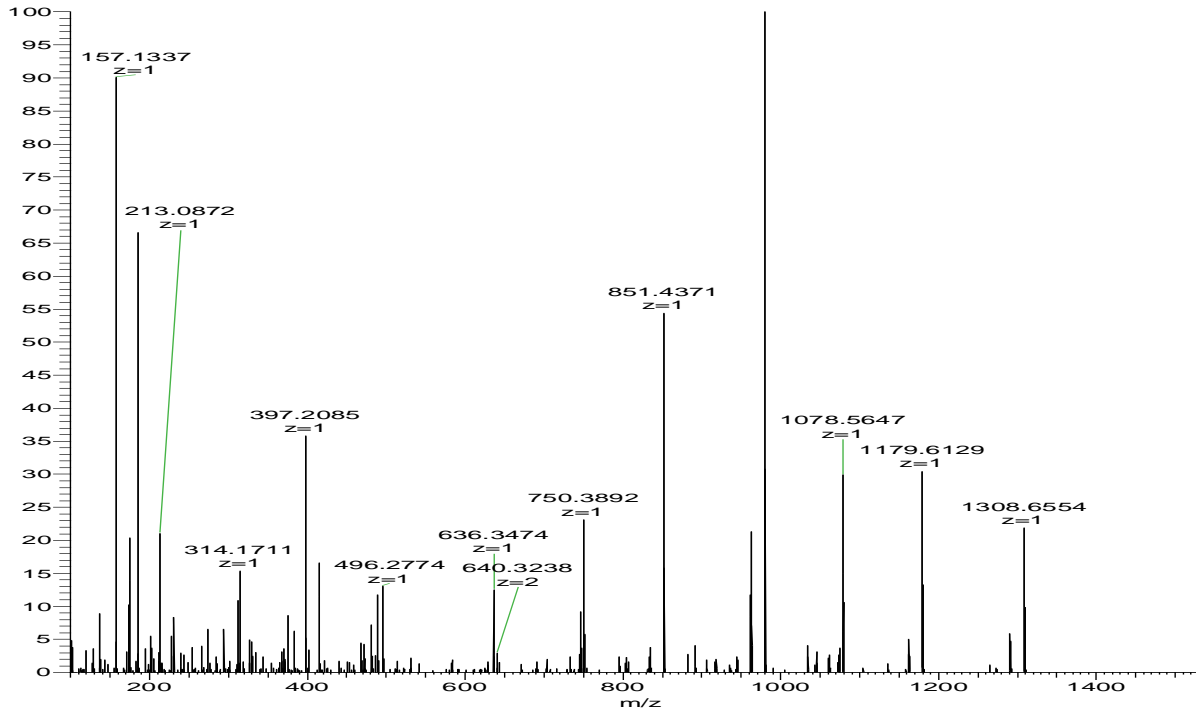
Cell lysis,  
Proteins  
extraction



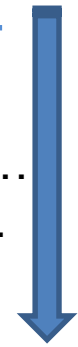
Sample Preparation,  
Trypsin digestion



20230403 WIB\_Zmays\_QEx\_CT0\_2\_3#11349 RT: 41.37 AV: 1 NL: 4.38E5  
T: FTMS + p NSI d Full ms2 746.8923@hcd27.00 [100.0000-1545.0000]

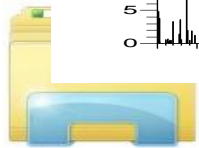


Prot...  
Arg...



Quant  
Associated  
Cellular

List of  
peptides



Protein  
Identification Tool

# Data processing...

Proteomics data processing:

**Proteins database !!!**



Major hurdle in rhizosphere microbiote characterisation:

**Most microbial species are unknown!!!**

30-40.000 species in soil

Soil bacteria are frequently not cultivable

Genomes are not available for most of them



One of the most frequently used option → metaTranscriptomics / metaGenomics

**Sequencing cost**  
**[gene] ≠ [protein]**  
**Incomplete genome...**

Sequence translation

Proteins/peptides DB





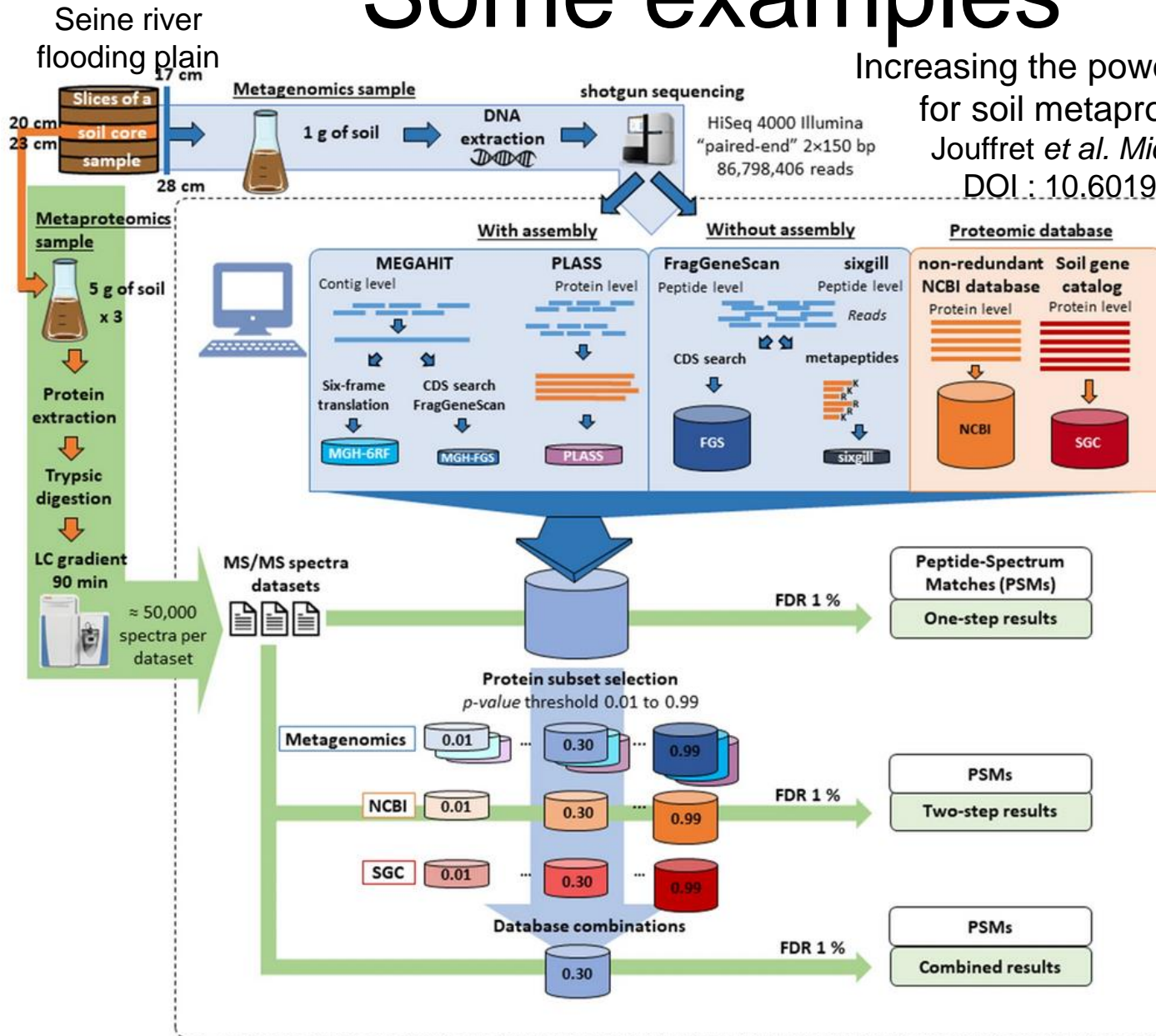
# Some examples

Increasing the power of interpretation

for soil metaproteomics data.

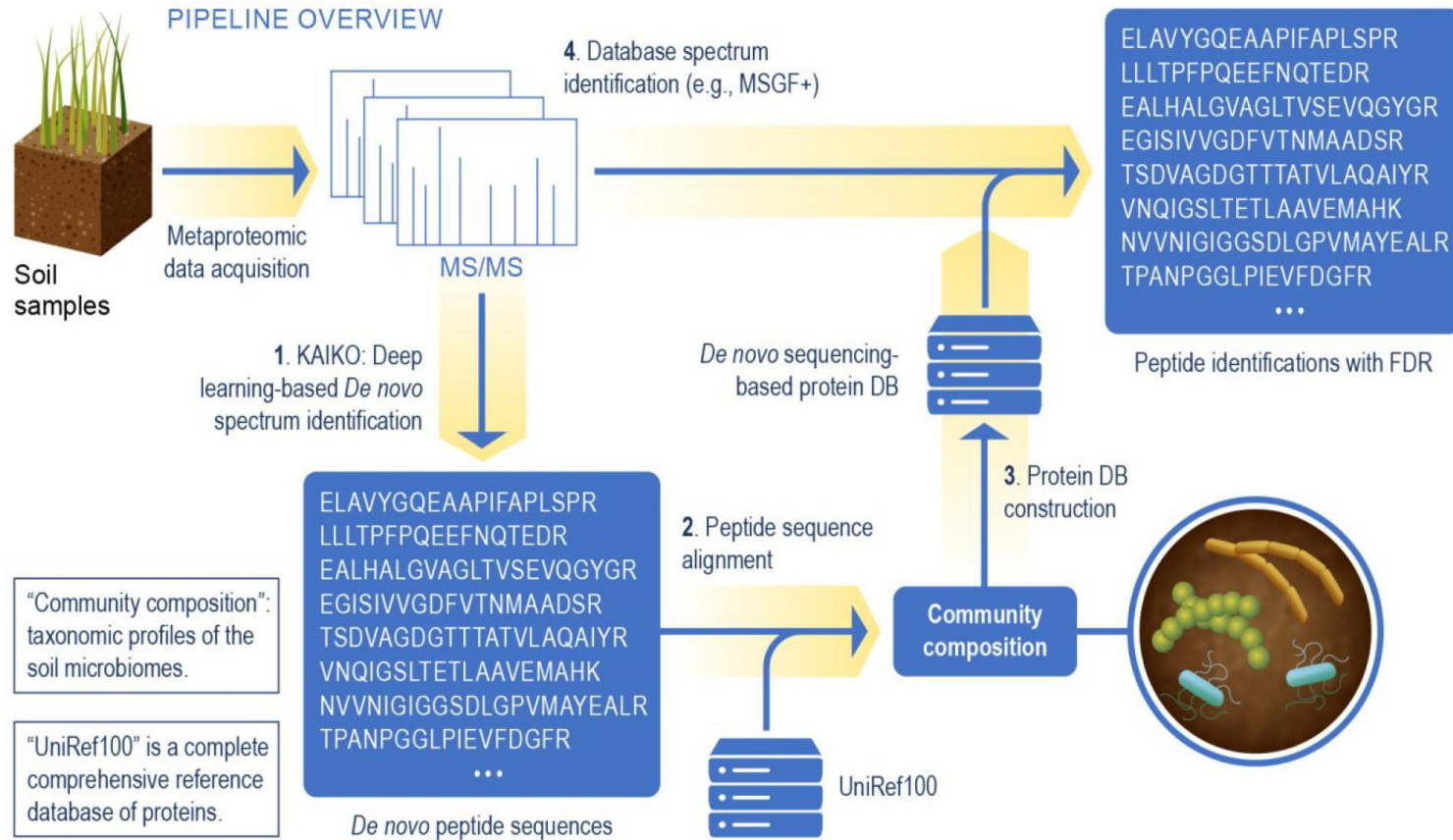
Jouffret *et al. Microbiome* 2021;

DOI : 10.6019/PXD026798



Workflows assessed for metaproteomics analysis of environmental samples

# AI approach



Overview of the metaproteomics data analysis leveraging *de novo* spectrum identification based on the Kaiko model.

Uncovering Hidden Members and Functions  
of the Soil Microbiome Using *De Novo* Metaproteomics.

Lee *et al.* *J. Proteome Res.* **2022**, DOI: 10.1021/acs.jproteome.2c00334

# PAPPSO team member's

