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# Development of a multimodal approach to study plant-microorganisms interactions using mass spectrometry imaging, Raman imaging and non-targeted metabolomics



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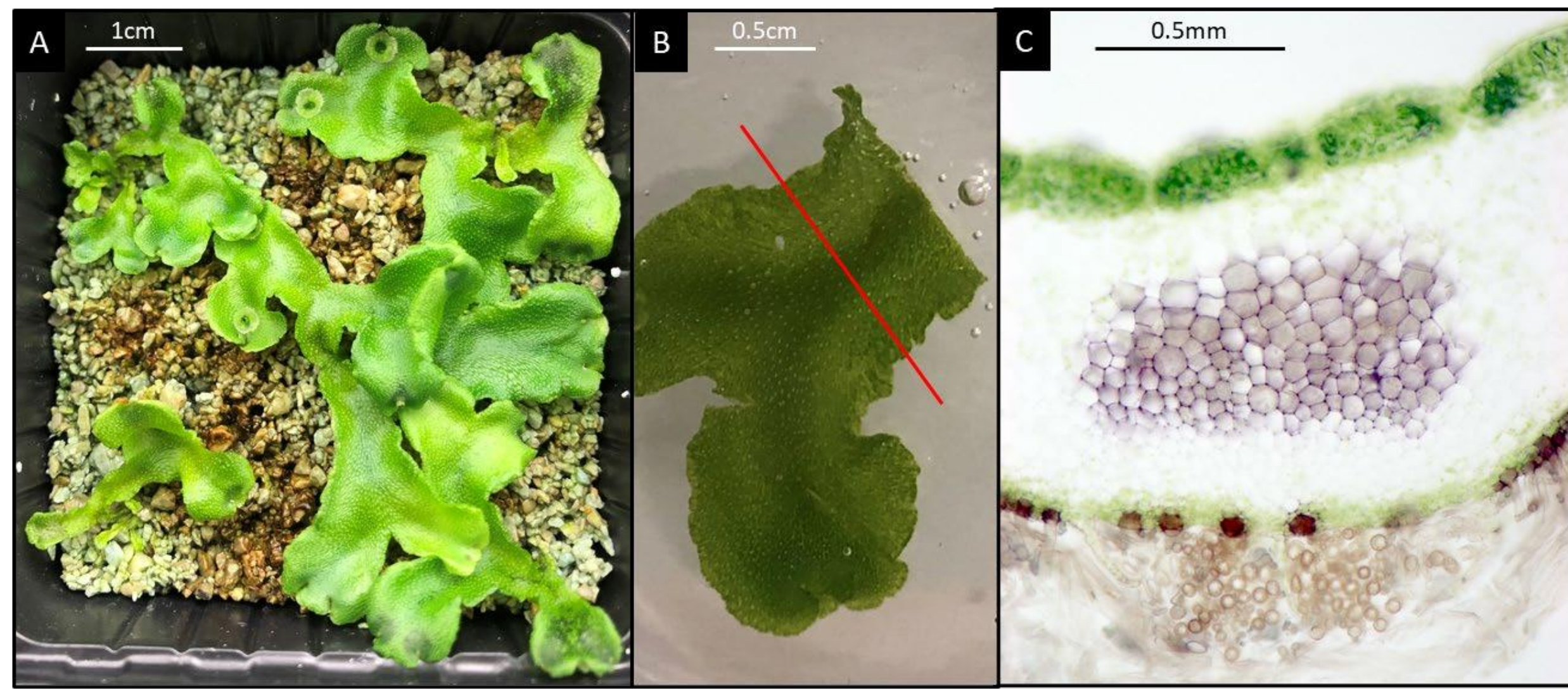
## Introduction

450 million years ago, an essential symbiotic relationship called **arbuscular mycorrhizal symbiosis (AMS)** was formed between plants and glomeromycota fungi<sup>1</sup>, enabling the first land plants to colonize the soil<sup>2</sup>.

It begins with a molecular dialogue between partners located in the soil, leading to the development of specialized fungal structures in the roots, called **arbuscules**, facilitating the exchange of nutrients<sup>3</sup>.

Understanding this metabolic dialogue is a challenge, but innovative techniques such as LC-HRMS and MALDI\* mass spectrometry imaging enable a complementary localization and quantification of metabolites in AMS. The integration of stimulated Raman microscopy enhances our understanding at the cellular level in a non-invasive way.

The first results of this study concern *Marchantia paleacea* mycorrhized by *Rhizophagus irregularis*.



A : *Marchantia paleacea*  
B : Thallus embedded in agarose 7%  
C : Transverse section (100µm thickness using vibratome) with arbuscular mycorrhizal fungi (purple area)

## Untargeted Metabolomics



### Data acquisition

by U-HPLC-HRMS (Thermo Scientific)

UHPLC : Vanquish with CAD and DAD detectors

MS : Q-Exactive Plus ESI +/-

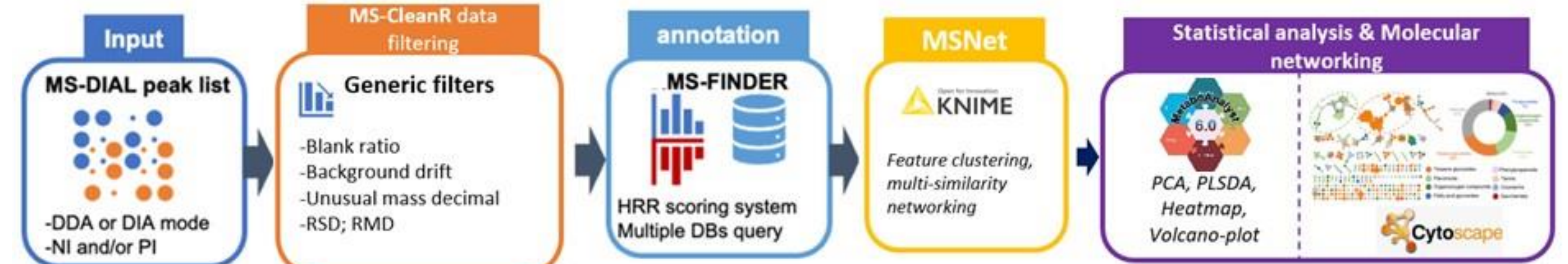
Biphasic extraction following MetaToul AgromiX protocol (MTBE, MeOH, H<sub>2</sub>O).

Lipidomic analysis  
(apolar compounds)

C18 analysis  
(semi-polar compounds)

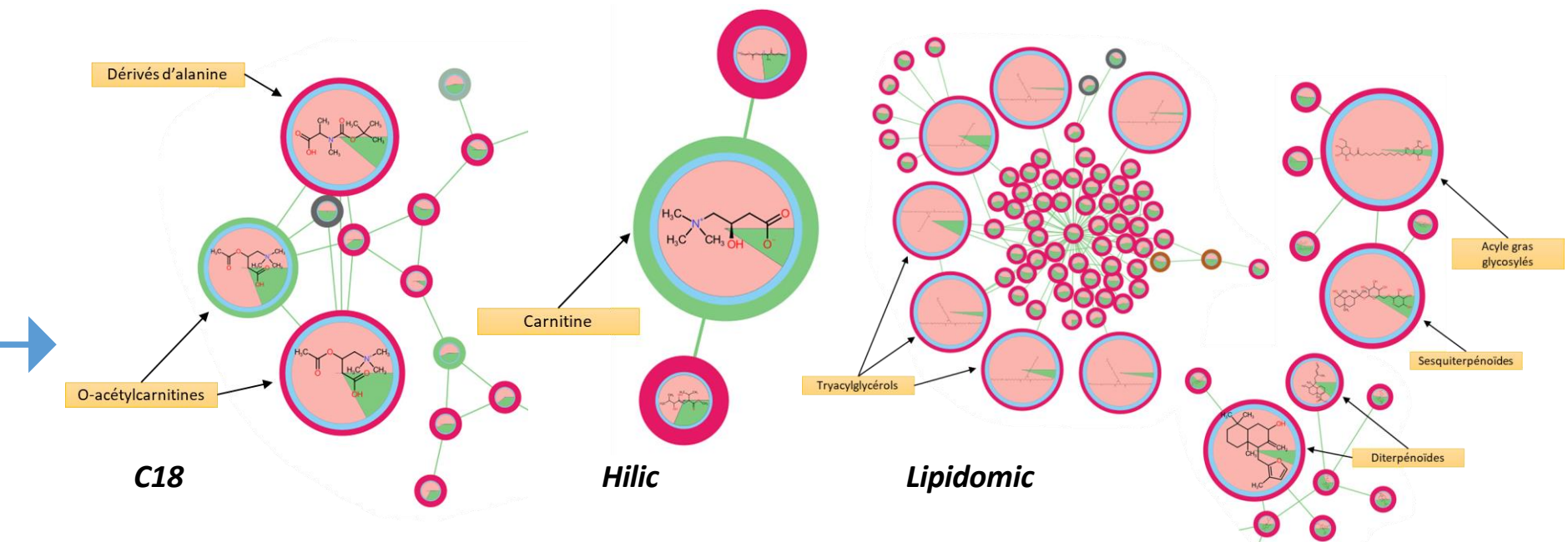
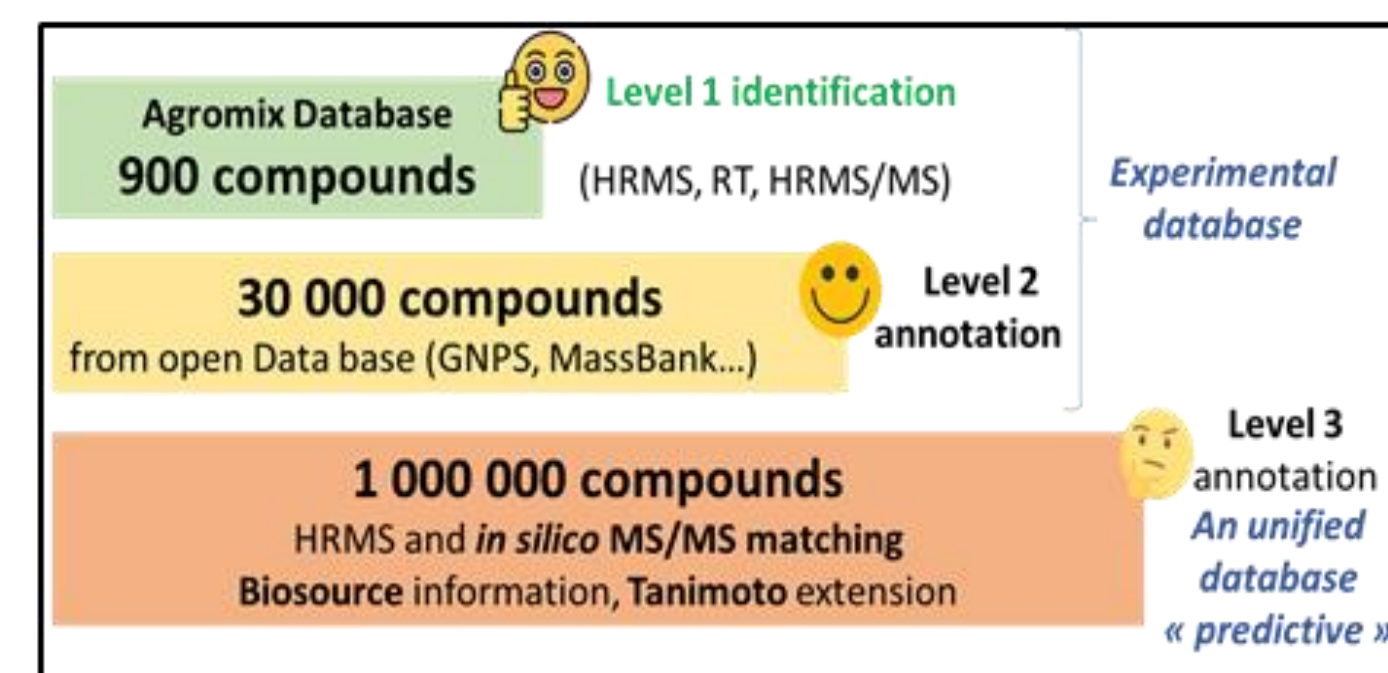
Hilic analysis  
(polar compounds)

### Data processing



### Peak annotation

with dedicated bioinformatic workflow for global metabolomics



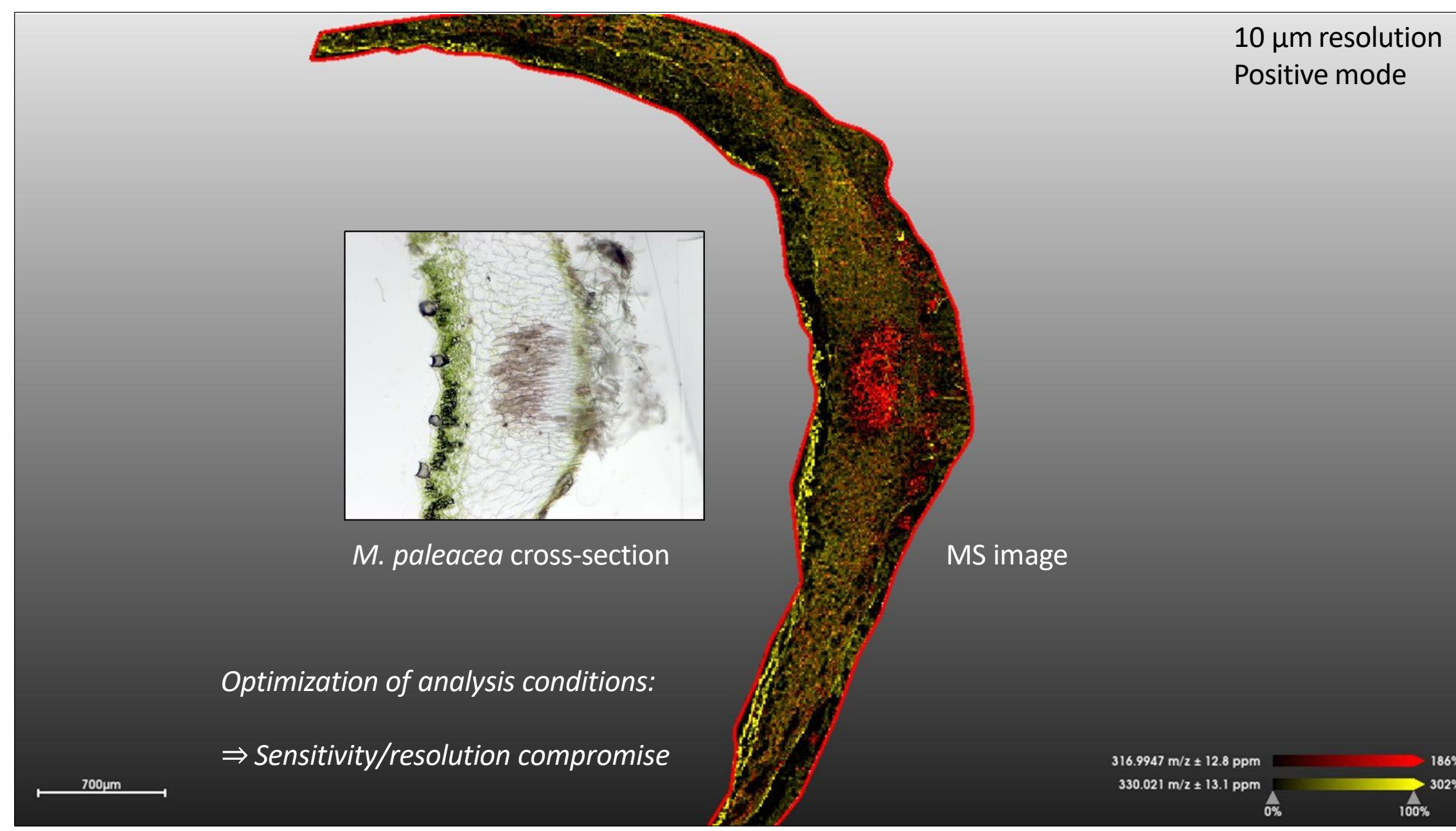
**Biomarker identification, lipids involved in Arbuscular Mycorrhizal Symbiosis?**

## Mass Spectrometry Imaging



### Data acquisition

with MALDI-MSI\* (timsTOF flex - Bruker)



### Sample preparation:

- MALDI matrix : DHB
- Deposition with the HTX TM3 sprayer (HTX technologies LLC)

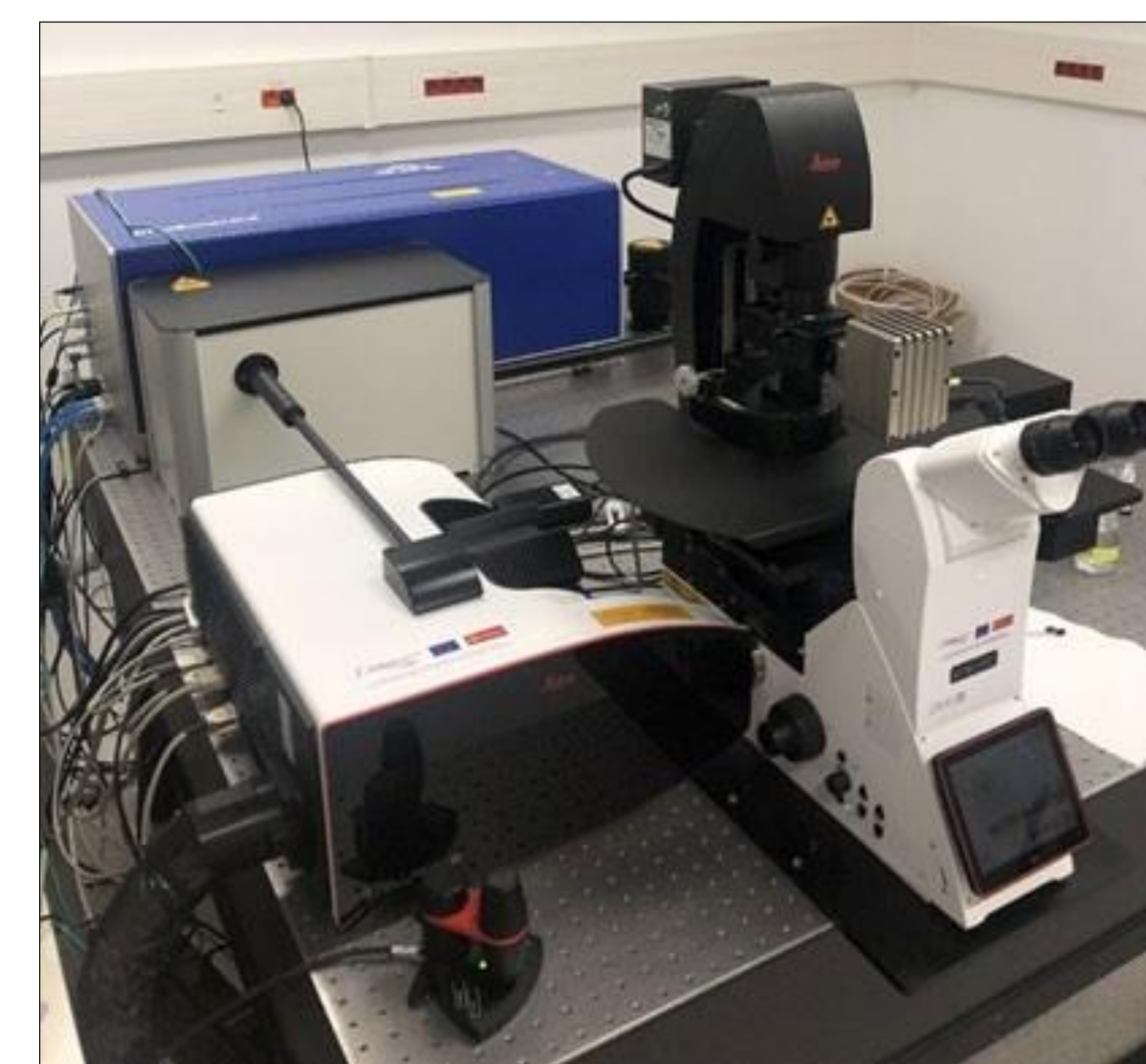
Hey ! Scan me and download the file to see 3D PCA of discriminative segmentation !

RED = Non mycorrhized area ions  
GREEN = Mycorrhized area ions  
BLUE = Centre of mycorrhize ions

\*Matrix-Assisted Laser Desorption-Ionization

⇒ **Targeting of biomarkers identified by LC-HRMS**

## Raman Stimulated Microscopy



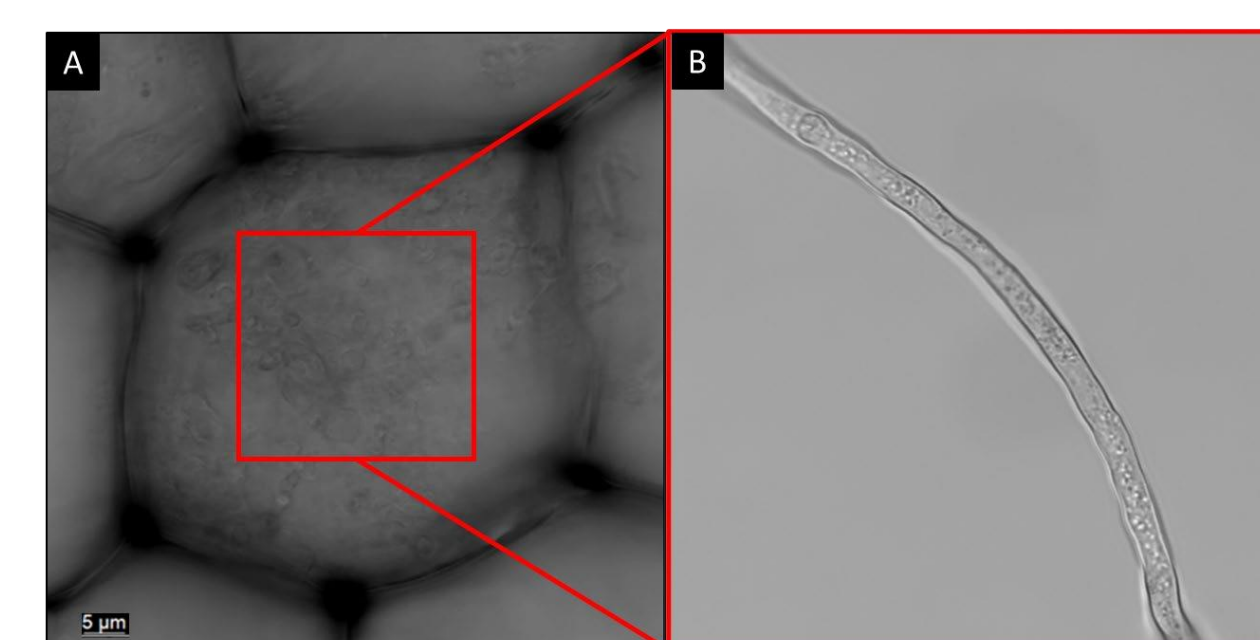
### Data acquisition

with Raman stimulated microscopy (STELLARIS 8 CRS - Leica)

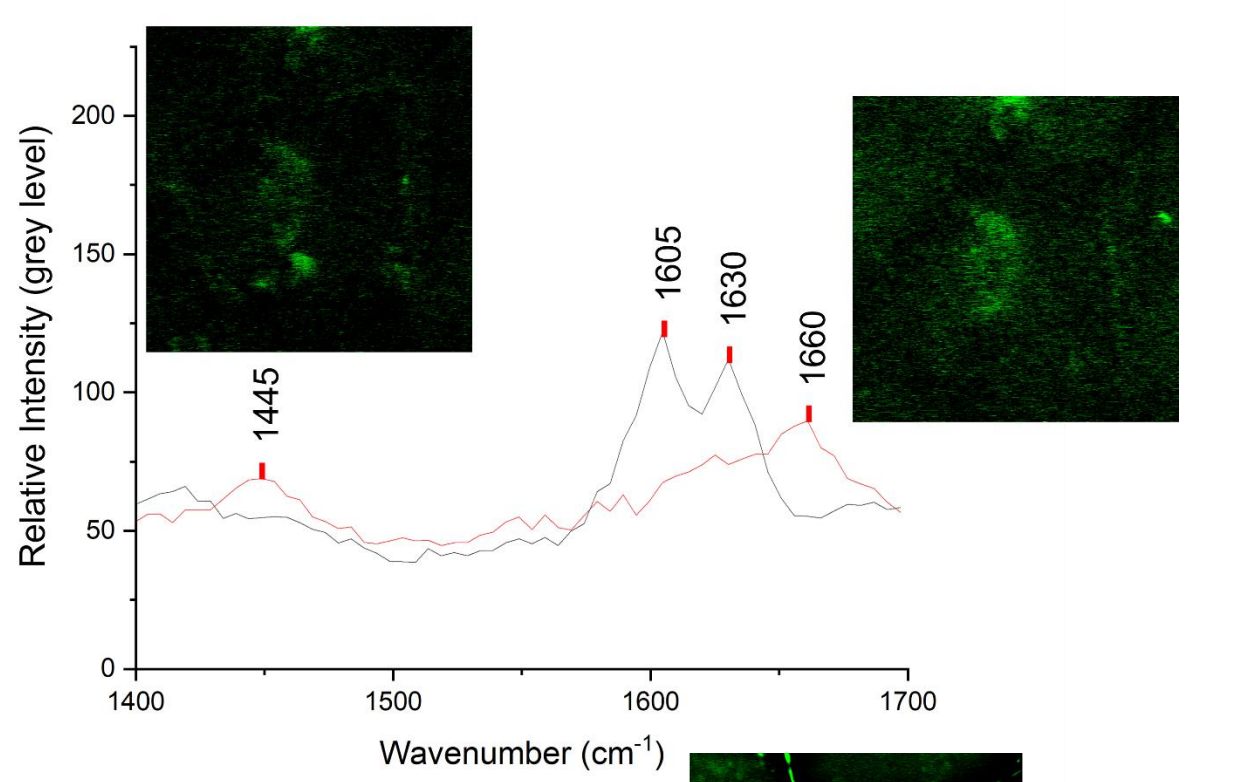
⇒ **Identification and mapping of Raman signals characteristic of biomarkers identified by LC-HRMS (single cell and hyphae scale) with tunable OPO laser.**

Optimization of analysis conditions:

- ⇒ Magnification
- ⇒ Number of accumulations
- ⇒ Signal averaging



Hi again ! Scan me to see real-time vibrations signals from laser excitation !



- (A) Raman spectrum of a non-mycorrhized cell of *M. paleacea* between 1400 and 1700cm<sup>-1</sup>. (I 35 % ; SRS gain 100 ; accumulation 2)
- (B) Raman spectrum from the hyphae of *R. irregularis* between 1400 and 1700cm<sup>-1</sup>. (I 35 % ; SRS gain 88,5 ; accumulation 1)
- (C) Raman spectrum from the inside of a mycorrhized cell of *M. paleacea* between 1400 and 1700cm<sup>-1</sup>. (I 30 % ; SRS gain 100 ; accumulation 1)

⇒ Two main peaks around 1445cm<sup>-1</sup> and 1660cm<sup>-1</sup> which could be respectively attributed to the signal emitted by saturated fatty acids and unsaturated fatty acids.

⇒ The difference in intensity between non-mycorrhized and mycorrhized cells could be explained by the presence of arbuscular mycorrhizal fungi.

## Conclusions

These results provide a great example on how multimodal approaches can help visualizing biological interactions between different organisms in a more constitutive way:

- Global LC-HRMS analysis helps **unravelling the metabolic segregation** occurring during AMS establishment.
- MALDI imaging helps localizing the metabolites involved at the tissue scale with **high sensibility level**.
- Despite not being as resolute as MALDI, the **high resolution** provided by Raman stimulated microscopy helps us to localize metabolites families. Allowing us to better quantify exchanges occurring during AMS formation.

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