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Dynamics of bacterial and fungal rhizosphere communities during the infection of *Brassica rapa* roots by the protist *Plasmodiophora brassicae*

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This work put the bacterial and fungal diversities in relation with *Brassica rapa* – obligate parasite *Plasmodiophora brassicae* (clubroot disease) interaction in the rhizosphere (Fig 1).



Diversity analysis allow the identification of potential key microbial players during the disease development

A GROWTH CHAMBER POT-EXPERIMENT

A split-plot experimental design

- 240 *Brassica rapa* cv. *Granaat* cultivated in a silty-clay soil and inoculated (I) or not (NI) with *P. brassicae* strain *eH*
- Growth chamber conditions: 16h light, 8h dark, 22°C/19°C
- 3 pools of 4 plants phenotyped at T1 (inoculation), and 7 (T2), 14 (T3), 23 (T4), 35 days (T5) after inoculation
- Plant traits : hypocotyl section and disease index

Microbial communities analysis

- Sampling of rhizosphere soil (RS) and bulk soil (BS) from inoculated (I) and not inoculated (NI) plants at each sampling date between T1 and T5
- DNA extraction following by a 16S and 18S rDNA amplicons sequencing performed by Illumina MiSeq platform
- Base trimming, assembly with a minimum of 30 bases overlapping - 100% homology between reads
- Strict dereplication, clustering (97% similarity) and taxonomic assignation (SILVA databases) (GnS-PIPE workflow)
- Statistical analysis with Bioconductor package and *edgeR* under R

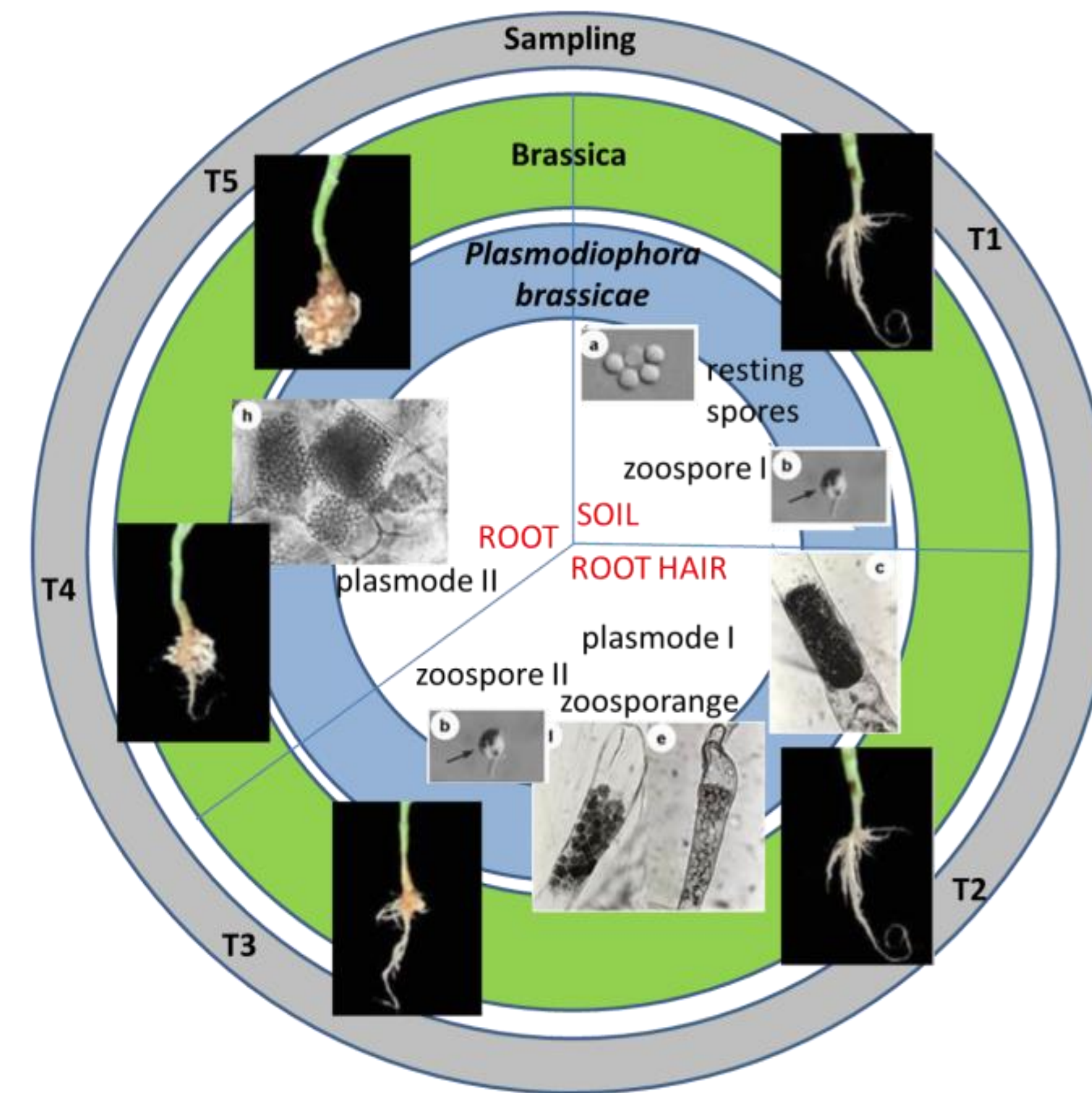


Fig1. Life cycle of *Plasmodiophora brassicae* (blue circle), the pathogen that causes clubroot, in relation with *Brassica* phenotype (green circle). The sampling strategy for plant trait measurements and molecular ecology is indicated in the grey circle

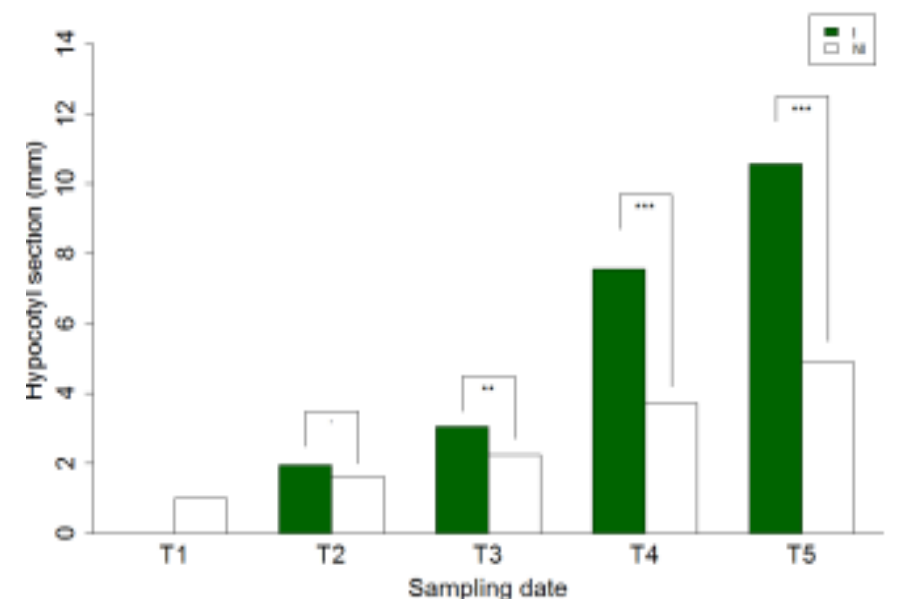


Fig 2 . Evolution of hypocotyl section from not inoculated (NI) and inoculated plants (I) between T1 to T5

KEY RESULTS

Plant traits related to disease development

- Differences of hypocotyl section from inoculated (I) and not inoculated (NI) plants starting 14 days after inoculation (Fig 2).
- Disease index of 15% at T3, 70% at T3 and 90% at T5 suggesting high sensibility of *B. rapa* cv. *Granaat* to clubroot.

Bacterial and fungal communities diversity evolved during disease development

- Means of 10000 and 45000 reads clustering in 10000 and 1600 OTUs for bacterial and fungal communities, respectively.
- Spatial (BS versus RS) and temporal effects on microbial diversity (Fig 3 and 4).

Key rhizosphere bacterial taxa identified

- Among 416 genus, the frequencies of 90 genus were significantly modified between inoculated and not inoculated treatments at T5. The 8 main genus significantly modified belonged to the *Proteobacteria* and *Bacteroidetes* phyla (Fig 5).

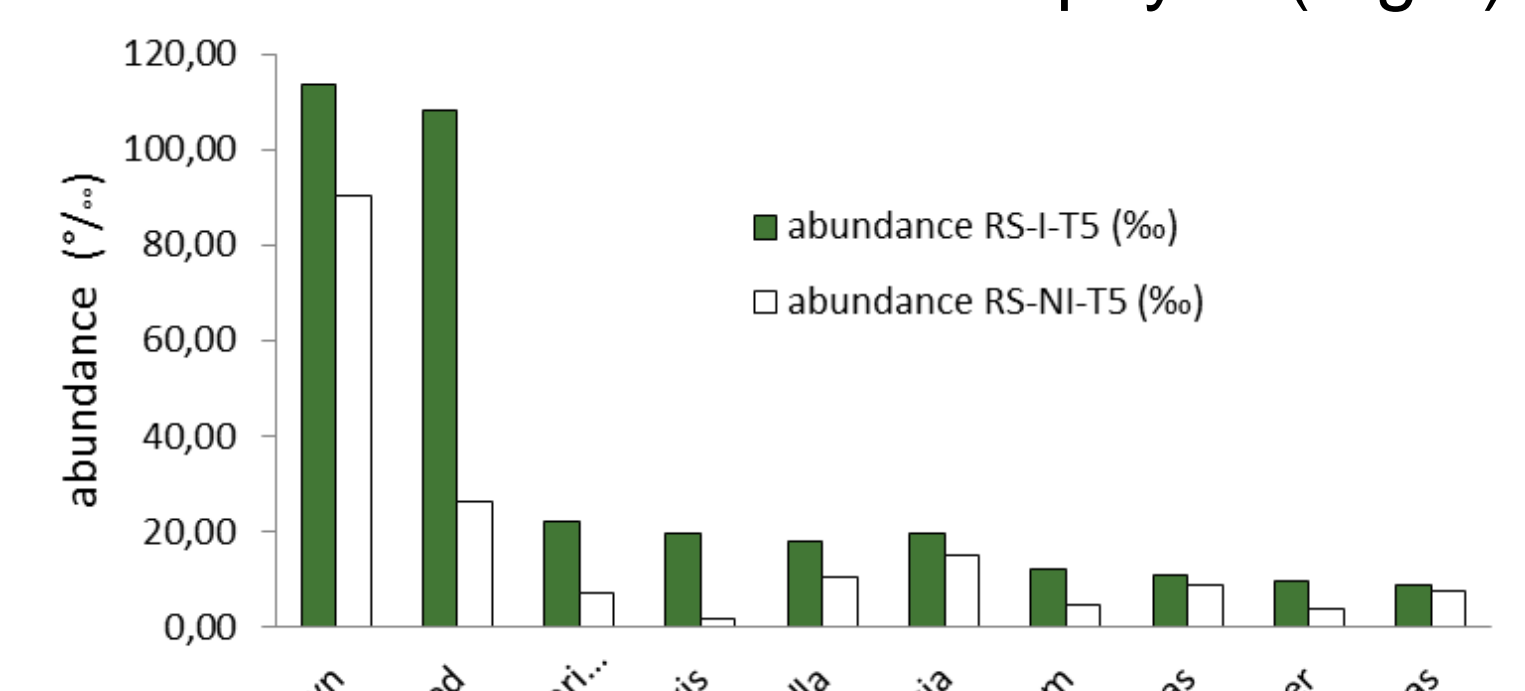


Fig5. Abundance of the 10 main bacterial genus whose frequencies were significantly increased at T5 in the rhizosphere of inoculated plants compared to healthy plants ($p < 0.05$)

Key rhizosphere fungal taxa identified

- Among 100 genus, the occurrence of 13 genus was significantly modified between inoculated and not inoculated treatments at T5 (Fig 6).
- At T5, the dominant fungus is *Olpidium* sp. which represents 38 % and 10 % of total sequences in the healthy and diseased rhizosphere, respectively.

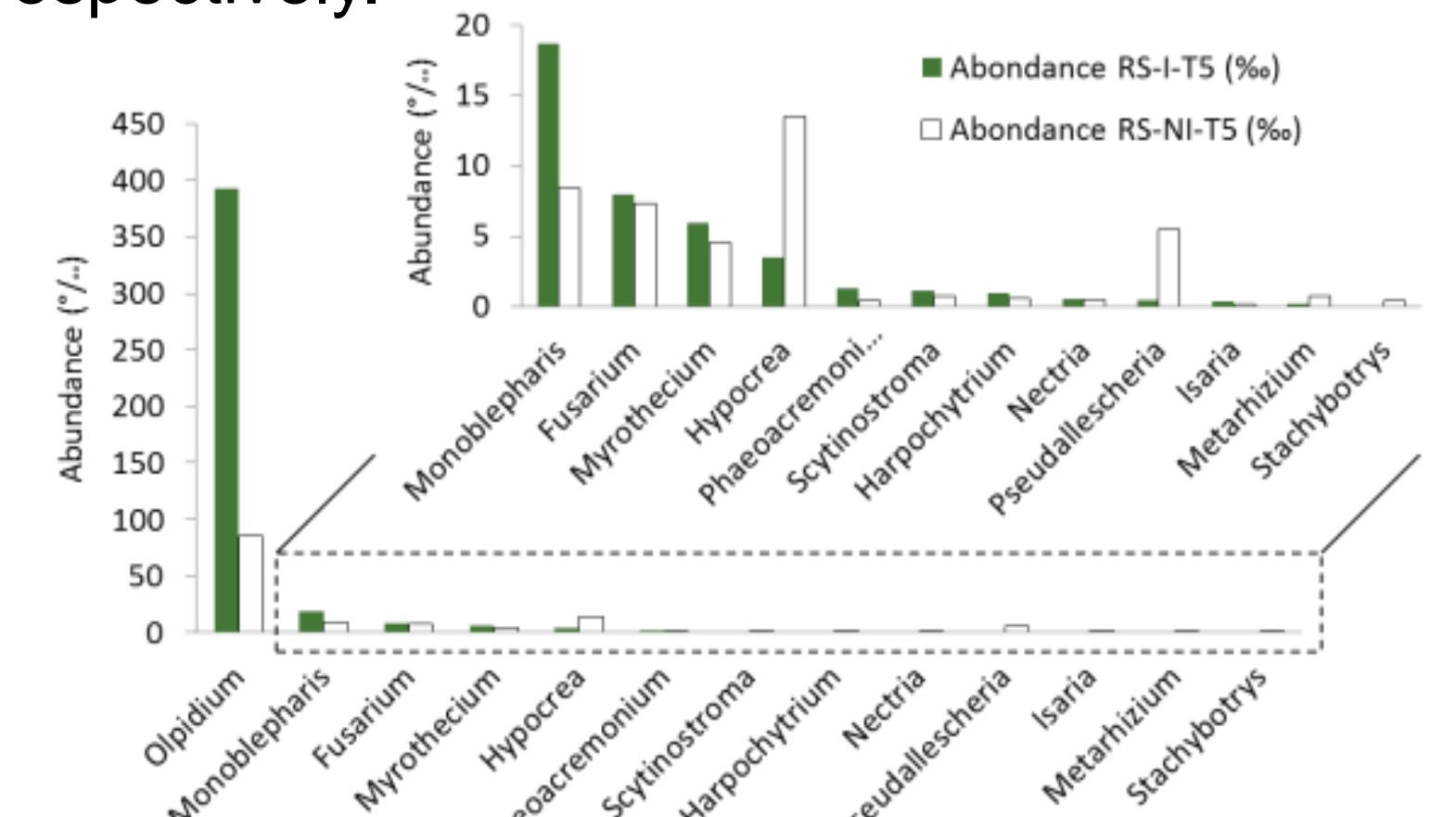


Fig6. Abundance of the 13 fungal genus whose frequencies were significantly modified at T5 in the rhizosphere of inoculated plants compared to healthy plants ($p < 0.05$)

TAKE HOME MESSAGE

- First description of the dynamics of bacterial and fungal communities during clubroot development.
- The strongest difference between microbial diversity appeared at the latest stages of disease development.
- The occurrence of bacterial genus belonging to *Proteobacteria* and *Bacteroidetes*, and the *incertae sedis* fungus *Olpidium* was strongly increased in the diseased plant rhizosphere when clubroot severity is higher.
- *Proteobacteria*, *Bacteroidetes* and *Olpidium* were host-specific rhizosphere genus able to develop intimate interactions with plant and seem to be key players of *Brassica-Plasmodiophora* interaction. Future directions will precise functional basis of these interactions.

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