POPmodels

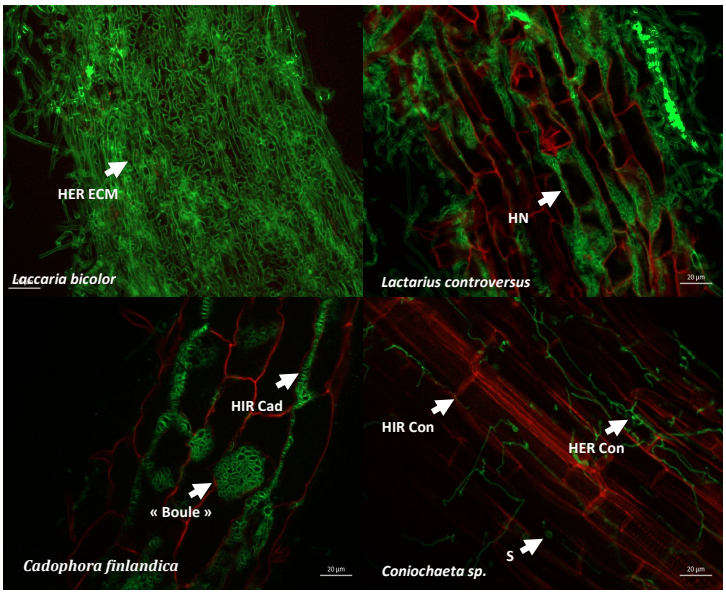

Figure 1: *Populus tremula x alba roots inoculated with an ectomycorrhizal fungus or an endophyte. Root cells were stained with propidium iodide (red) and fungal cells with WGA-Alexa fluor 488 (green).*

Understanding Poplar-Microbe Interfaces: From model systems to model synthetic communities

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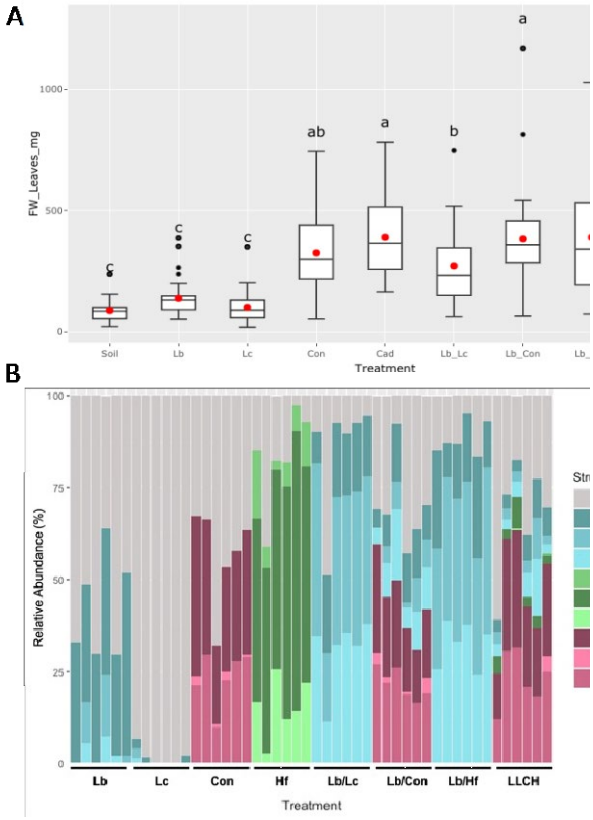
Thematic action concerned: *WP1*

Context —
Thousands of species of fungi occur in the soil, but only a small fraction is able to form mutualistic symbioses promoting tree growth. Ectomycorrhizal fungi (ECM), but also endophytes with positive, negative or neutral effects on their hosts, are becoming increasingly recognized as important members of the tree microbiome. Despite their importance for tree growth and resistance to stresses, we still know very little about the mechanisms by which these fungi associate and communicate among each other and with their host trees, or the mechanisms by which they contribute to ecosystem processes such as nutrient cycling, among others.

Objectives —
The current knowledge on the molecular signalling in ECM interactions was obtained with a few *in vitro* model systems, including our model *Laccaria bicolor*-Poplar. While significant progress was made in the elucidation of signals that are essential for ECM development, very few is known at the molecular level for endophytic interactions and the possible inhibitory or synergistic effects that these fungi may have on ECM fungi and towards the host tree.
To assess if common or different mechanism of interaction are found for different types of mutualistic plant-fungal interactions, one challenge will be to extend our molecular studies from simple *in vitro* systems to more complex and finally natural systems. In this project, we aim at determining whether plants differentially recognize beneficial microorganisms and, further, how do plants engage at the same time with a wide range of microorganisms?

Approaches —
In this experiment, we analysed the colonisation of naïve *Populus tremula x alba* roots by two ectomycorrhizal fungi and two endophytes either by single inoculation or by different combinations of dual and multiple inoculations. In sterile pots, 3-weeks old poplar were grown in 200 ml of gamma irradiated sterile soil plus 5% volume of fungal inoculum for 30 days. For each treatment, five pools composed of the arial parts or roots from five plants were harvested for molecular analysis and 6 roots were kept for microscopy analyses. In addition, soil samples from each pot were snap frozen in order to analyse the fungal transcriptome in the soil compartment. Fresh biomass was determined and stained root samples were observed by confocal microscopy (the root cell walls stained with propidium iodide (red) and the fungal cell walls with Alexa fluor (green)). Colonised roots, control roots and soil samples (for fungal mycelium controls) were used for total RNA extraction.
Due to the low RNA yields obtained, SMART technology for very low input sequencing library construction was tested for 10 representative samples.

Key results —



Aerial fresh biomass and root colonization of P. tremula x alba. *Aerial fresh biomass was determined during the harvest* ***(A)****. Relative abundance of fungal structures (%) within the root system of Populus tremula x alba WT between treatments after 30 days of growth. Laccaria bicolor (Lb), Lactarius controversus (Lc), Coniochaeta sp. (Con), Hyaloscypha finlandica (Hf), Laccaria bicolor/Lactarius controversus (Lb/Lc), Laccaria bicolor/Hyaloscypha finlandica (Lb/Hf), Laccaria bicolor/ Coniochaeta sp. (Lb/Con), all fungal strains (LLCH)* ***(B).*** *M mantle; HN Hartig net; HER extra-radicular hyphae; HIR intra-radicular hyphae.*

- Endophytes and co-inoculations (dual and multiple) between ECM fungi and endophytes showed a plant growth promoting effect. (Fig.2A)
- In our experimental set-up, ECM fungi formed ectomycorrhiza with *P. tremula x alba* root only when ECM fungi where in dual inoculation with either another ECM or an endophyte. (Fig.2B)
- Both endophytes, *Cadaphora finlandica* and *Coniochaeta* sp, colonized poplar roots but with very distinct appearances. *Coniochaeta* displayed highly melanized, thin extraradicular hyphae and spore-like structures, while *Cadophora* formed intercellular punctuated by globular structures (Fig. 1).
- Within our more complex system, all four strains formed interactions with poplar roots but at a low frequency except *Coniochaeta* which interacts with poplar roots at the same rate within each fungal combination. (Fig.2B)
- Very low input test RNA-Seq for 10 samples produced promising results. Analysis of the poplar transcriptomes but also for most of the fungal transcriptomes were possible (Table 1).

Main conclusions including key points of discussion —

- ECM fungi formed ectomycorrhiza with *P. tremula x alba* only in combination with either a ECM or an endophyte and different colonization patterns were observed within ECM/endophyte dual inoculation. Their ability to colonize the roots could be due to synergistic or competition mechanisms between fungi or by their growth rate which allowed them to get access to the root system before the other.
- The two endophytes showed very distinct colonization patterns. *C. finlandica* alone formed intra-radicular structures while *Coniochaeta* hyphae stayed mainly in the surrounding of the root as an epiphyte. This could mean that two fungi belonging to the same guild are able to form different kind of interactions depending on the environmental conditions.
- Ectomycorrhizal (in dual inoculation) and endophytic interactions provided growth benefit to their host unrelated to their colonization pattern. This suggests that the growth promoting effect observed could be due to the direct nutrient transfer at the plant/fungus interface but also to an easier access for the plant to the nutrients from the rhizosphere ensured by the fungus. Indeed, within the more complex system, fungal structures observed were mainly those of *Coniochaeta*. Poplars would be associated preferentially with this fungus which provides it growth benefit without any carbon cost. This suggests that the establishment of a plant/fungus interaction would depend on the plant perception mechanisms but would be also related to the nutritional balance.

Sample	paired reads	mapped reads Poplar	mapped reads Hyaloscypha	mapped reads Laccaria	mapped reads Lactarius	mapped reads Coiochaeta
ControlRoot4	71 626 942	56 163 320				
HfRoot3	69 904 626	43 905 152	8 308 348			
LLCHRoot6	70 734 330	51 421 320	588 684	1 225 644	48 658	725 526
LbRoot4	81 294 236	58 576 756		3 460 698		
LbConSoil4	76 058 014			1 337 314		27 248 126
P20Lc1	68 210 012				46 214 862	
P20Lb2	67 950 032			48 674 434		
LbConSoil1	60 693 830			896 032		25 945 148
LbHfRoot6	78 906 054	47 465 864	1 329 102	11 384 938		
LbConRoot6	72 494 648	48 783 580		6 547 172		472 388

Table 1 Summary of mappings against the poplar reference genome as well as against the four fungal references.

Perspectives —

Due to the promising RNA-Seq results for 10 test samples, all 90 remaining RNA samples were sent for low input sequencing. Results are expected for End of March 2024.

Valorization —

Presentation of first results in lab seminars, to collaborators of the PMI project and at the annual LABEX Arbre meeting in November 2023. These results have been also submitted for either a poster or oral presentation to ICOM12 (International Conference on Mycorrhiza), to be held at Manchester in August 2024.

Leveraging effect of the project—

The POPmodel project is closely linked to our international long-term project Plant-Microbe Interfaces (PMI; https://pmiweb.ornl.gov/; since 2010). The intermediate results presented here were already discussed in the frame of PMI and were considered for future directions of the project.