Impact of abiotic stresses on ectomycorrhizal symbiosis and role of ectomycorrhization on tree responses

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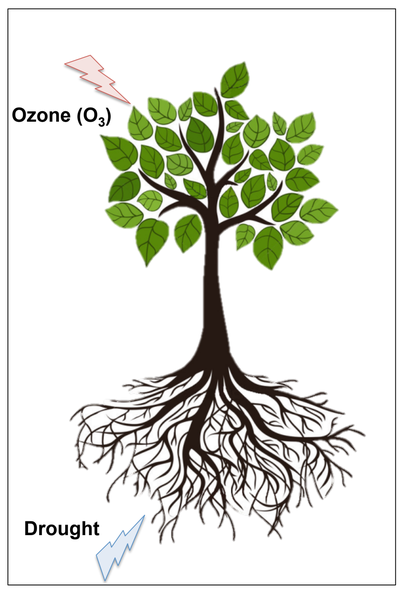
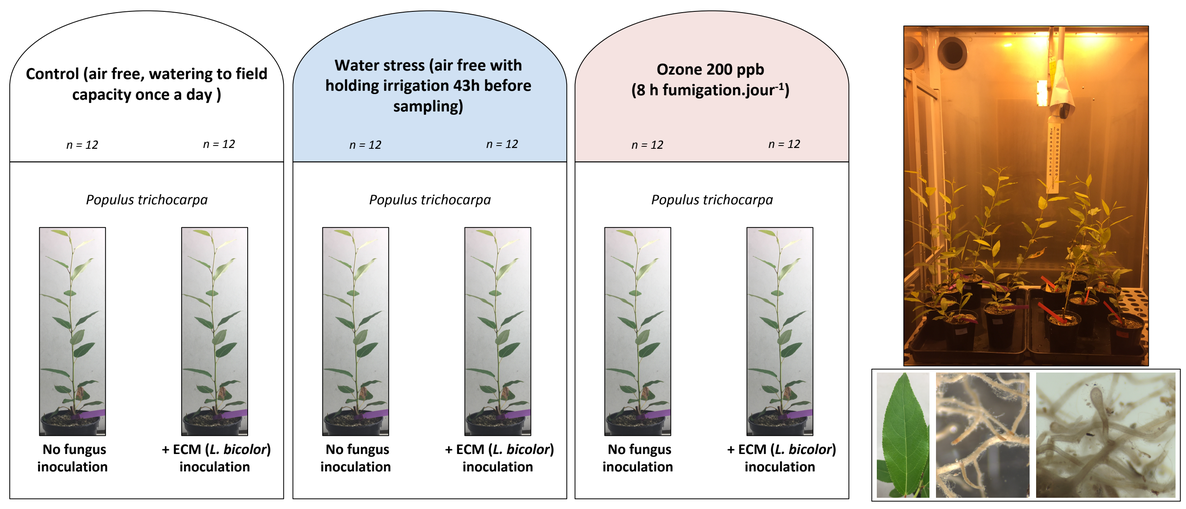
Context & Aims



Long-lived and stationary organisms such as **trees** are subjected to a wide variety of environmental cues from both **biotic and abiotic origins**. With the on-going **climate change**, more severe and recurrent abiotic stresses are expected. It is crucial to address the following questions of i) how trees interact with their biotic and abiotic environments, ii) does the ectomycorrhizal symbiosis mitigate the impact of stress at a systemic scale and iii) does abiotic stresses challenge the ectomycorrhiza (ECM)-tree interaction. Here, we tested the hypothesis that ECM trigger a deep rewiring of the hormonal network, which in turns could modify the early perception of abiotic stresses, sensed either by the root or the shoots.

We aim to better understanding the molecular plasticity in the above and below part of **poplar (*Populus trichocarpa*) trees under different environmental cues: drought and ozone.** In this context, the specific objectives are to decipher the impact of these abiotic stresses on (i) metabolites, (ii) hormonal and (iii) **transcript signatures** of *Populus* leaves and ECM or no-ECM roots as well as the impact of ectomycorrhization on tree responses to stress.

Material & Methods

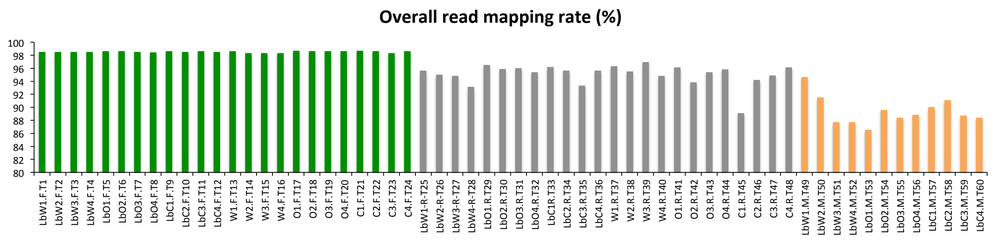


-Plant growth conditions**:* Cuttings were rooted for 2 weeks in distillated water. Young rooted poplar were planted in attapulgite clay and half of pots were inoculated with an ECM-fungus, *Laccaria bicolor*. Each pot was given a dilute nutrient solution weekly and kept under climate-controlled greenhouse conditions, maintaining a 16-h photoperiod and a temperature of 22°C (day) during three months **- *Exposure conditions: Homogeneous plants were set in phytotron chambers during for two weeks, prior to stress exposure (200 nmol.mol^{-1} O_3 for 8 hours or withholding watering for 43h). Non-inoculated and inoculated trees were randomly assigned to one of environmental conditions, and control plants harvested together with stress plant. **- *Sample**:* Leaf, lateral roots and/or ECM roots were harvested separately and frozen at -80°C for further RNA extraction and lyophilized for metabolites and hormones (GC/MS-MS) profile analyses. A pool of four plants was used for each repetition and each treatment.

Results

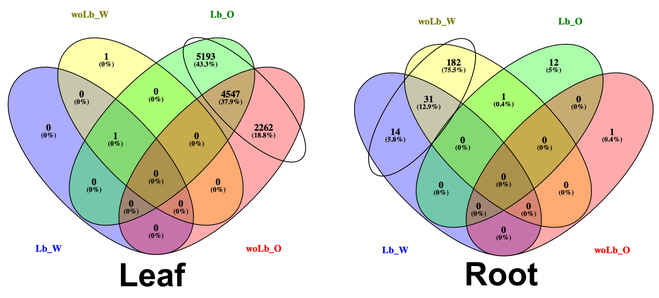
- **Physiological measurements** (Leaf gas exchange, root respiration, RWC, PO, plant biomass, etc.) ➡ No impact of ECM or short-term stress on whole plant functioning.

- **RNA-seq:** 60 mRNA libraries were sequenced (Hiseq3000, GenoToul, France). Raw reads were filtered, trimmed and aligned with the *P. trichocarpa* genome version 3.1 (Phytozome, JGI) or *L. bicolor* (Mycocosm, JGI*)*.



Read mapping against Poplar reference genome covering more than 89% of our read sequence samples.

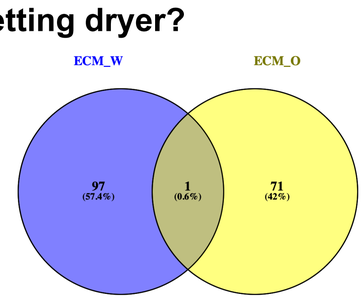
Is there an impact of ectomycorrhization on tree responses (in leaves and roots) to ozone or water stress?



The transcriptome of root and leaf tissues were responsive to stress exposure (p>0.05, Deseq2). Venn diagrams compared the response of Poplar inoculated with *L. bicolor* (Lb) or not (woLB). *(Ozone-treated versus control* = Lb_O ; woLb_O; *Water-stressed versus control* = Lb_W ; woLb_W)

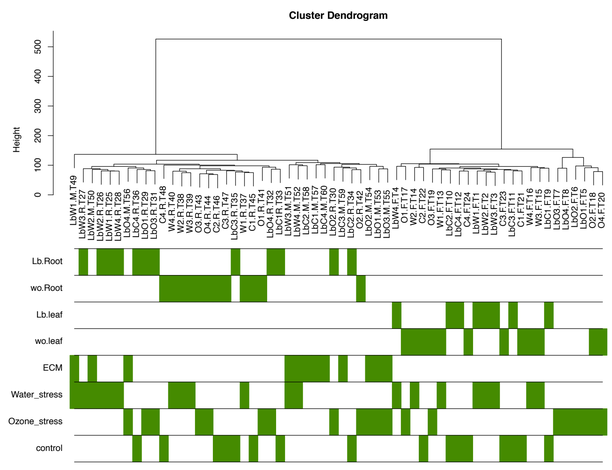
Ozone application strongly impacted the leave transcriptome while the response to soil **water stress responses preferentially occurred within roots,** regardless of the plants being inoculated with an ECM fungi or not. Those responses triggered GO enrichment in terms, such as **“response to abiotic stress”**, **“responses to endogenous stimulus”** or **“response to stress”**, highlighting that early stress signaling occurred within stressed organ, with a little systemic impact.

Do ECM roots sense that poplar leaves are exposed to O_3 and that soil is getting dryer?

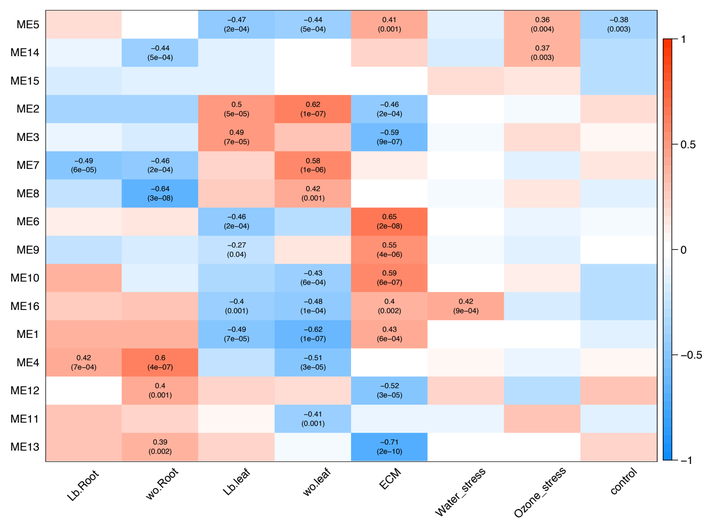


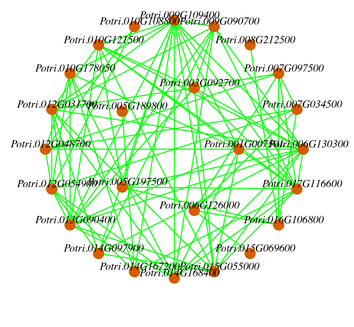
The poplar transcriptome of ECM was poorly responsive to stress exposure. Regulation of gene expression in **ECM appeared distinct during ozone or water stress responses.** Only one gene is showed as common to stress (Transducin/WD40 repeat-like superfamily protein).

Topology-based gene network of “Omics data”



Samples clustering based on the expression of 30,895 poplar genes. Our experimental design is summarized by the below Heatmap (green= include, white= not include). Based on topological overlap within the gene matrix, a signed network is constructed using WGCNA. Sixteen modules were delineated among the whole sample- normalized gene expression matrix, driven by both organs or treatments.





Adjacency-based gene network within Module 14 Top-connected gene are showed in the center

Heatmap provides the correlation between eigengene of each module (ME) and our experimental set-up. Color-code is the Pearson coefficient, Student asymptotic p-value is given in bracket. WGCNA modules will be associate with metabolomics and hormonomics data to access the biological significance of the transcriptional network.

- On-going experiments of phytohormone and metabolite profiles will be linked to RNA-seq data.

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