BRAWO (Intermediate report)

Fate of lignin altered by brown and white rot fungi

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Context — In forest ecosystems, white and brown rot fungi are the main actors of organic matter recycling and more particularly lignocellulosic biomass. Indeed, they have abilities to degrade or alter lignin to favor access to wood polysaccharides. The strategy used by white rot fungi is based on extracellular enzymatic system able to degrade both lignin and polysaccharide. Brown rot fungi use another strategy based on hydroxy radicals production leading to polysaccharide degradation and generating chemical modification in lignin. The differences between these two strategies come from fungal evolution history. Indeed, the emergence of brown rot would be due to a loss of genes coding for functions involve in lignin degradation into several white rot fungi. These events would be produced several times during fungal evolution history. Moreover, the fact that brown rot fungi dominate coniferous forest could come from an advantage in relation to white rot fungi.

Objectives — In this project, we want to understand how strategies used by white and brown rot fungi impact the microbial diversity and carbon persistence in forest soil. Secondly, we want to test the following hypothesis: brown rot fungi have an energetic advantage in relation to white rot related to evolutionary history.

Approach — We made several experiments based on wood incubation in common garden located in Breuil-Chenue forest (Morvan). The objective of the first one is to understand the impact of several degradation stages caused by *Gloeophyllum trabeum* on associated microorganisms and carbon persistence in soil forest. The second experiment was made to compare impact of both strategies used by white and brown rot fungi on associated microorganisms and carbon persistence in soil forest. The last experiment tests the theory of the "home field advantage" known for litter but not for wood substrates. Two methodologies are used i) omics tools to get functional data (metatranscriptomics) or diversity (metabarcoding) of associated microorganisms, and ii) biochemical methods to follow wood composition overtime.
In parallel, we perform *in vitro* experiment based on cultivation of white and brown rot fungi on beech sawdust. Several parameters are monitored: respiration, fungal biomass production, wood composition and production of extracellular enzymes production. All parameters will be fitted in mathematical model to predict carbon flux.

Key results — Results presented in this section correspond to methodological milestones that have been reached:

In situ experiments:

- Degradation of Norway spruce, beech and poplar by *Gloeophyllum trabeum* and *Trametes versicolo*

- Chemical composition of Norway spruce, beech and poplar
- Chemical composition of Norway spruce *degraded by Gloeophyllum trabeum*
- Extraction of gDNA from wood incubated in forest soil

In vitro experiment on energetic advantage:

- Monitoring of *T. versicolor* and *G. trabeum* respiration on beech sawdust during 3 month.

Main conclusions including key points of discussion — During our research, we faced some experimental problems:

- In situ experiments: The quality of RNA extractions from wood samples was not sufficient to do metatranscriptomics analyses. For this reason we decided to switch on DNA extraction in order to do metabarcoding. Moreover, when samples were collected, we observed few colonization of microorganism because this year was very dry. To avoid a similar problem, the collection of incubated samples initially programmed in November 2019 was extended for 6 months (May 2020).

- In vitro experiment on energetic advantage: At first, experimental conditions were tested and optimized on specific machine to follow fungal respiration. However, we had to deal with a challenge represented by keeping sterile conditions overtime, when regularly collecting gas in the incubators. To do that we used antibiotics to avoid bacterial contaminations but the effect was to strongly reduce fungal growth. Then, we find a solution, the use of filters with a very thin porosity. Although we optimized our conditions a failure of the gas analyzer occurred. We made another protocol to follow fungal respiration with a new gas analyzer using an infrared technology.

Future perspectives — First of all, two incubation of wood in Breuil-Chenue forest are still in progress. We plan to send 16S and ITS apmlicons in order to sequence them and get back data on microbial diversity. Then we will work on these data to write a paper. Secondly, we will determine parameters written above for the 3 month point. Moreover, we will do a kinetic for 15, 30 and 60 days and we will determine all parameters.

Valorisation —

Nicolas Valette, Eric Gelhaye, Gry Alfredsen, Barry Goodell, Delphine Derrien. **2019** The Home Field Advantage theory could be used for carbon sequestration and forest management ? Soil Organic Matter 2019 conference. Adéalïde, Australie. Poster.