



## *Molecular Ecology of Leaf Litter Decomposition*

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**Context** — Terrestrial carbon (C) is largely located in soils and a large part of the sequestered C in forest ecosystems comes from soil litter. The decaying leaf litter is colonized by bacteria, but mostly by leaf fungal decayers and also by ectomycorrhizal (ECM) fungi. Saprotrophic fungi are the main decomposers of coniferous and deciduous wood and litter. However, ECM fungi may be in competition with leaf decomposers for nutrient acquisition, particularly nitrogen (N). Indeed, being supported by carbohydrates from their host plants rather than depending on energy from organic matter, mycorrhizal fungi are very competitive and monopolise nutrients for themselves and their hosts. Mycorrhizal fungi could play a major role in the turnover of litter accumulated on the forest floor, that represents potentially the main source of N for plant growth, and then in the N cycling. But the interactions and successions of these two ecological guilds remain very little studied.

**Objectives** — The LeafLitterOmics project proposes an integrated field study of the succession of fungal species during litter degradation and a monitoring of associated microbial functions. The goal is to provide a better understanding of the links between organic matter chemistry, microbial community composition, and enzymatic activities expressed during litter decomposition. The proposed project will survey (1) the changes in litter chemical composition by spectroscopic techniques, (2) the composition of the microbiome by high-throughput rDNA sequencing, and (3) the study of microbial functions involved in the degradation of plant organic matter (oak litter) thanks enzymatic approaches. These experiments will be designed to define links between functional diversity, fungal ecological groups and litter chemistry / quality.

**Approach** — This research proposes to molecular genetic analyses of microbial community composition and activity to understand the fungal interactions and successions between leaf decomposers and symbiotic fungi. Leaves litterbags have been bring in a forest site for metagenomics, enzymatic and chemistry analyses. During autumn 2013, senescent oak leaves have been collected from two French sites: Champenoux forest (54) and Breuil forest (58). In January 2014, for each type of litter, thirty bags ("litterbags"  $9.5 \times 14.5$ cm, with a porosity of  $1.4 \times 1.8$  mm) were placed in the Champenoux forest (experimental site of the MOS network). The timemonitoring of the decomposition was carried out during 2 years. The aims were:

1. To survey changes in the composition of microbial communities (ectomycorrhizal fungi and leaf decomposers) during the degradation and stabilization of oak litter by using high-throughput rDNA sequencing;
2. To measure the expression of functions (enzymatic approaches) related to the degradation of organic matter and the mobilization of certain elements (C, N, P). (3) To assess changes in the leaf litter chemistry during the decomposition by biochemistry analyses.

**Key results** — The kinetic of litter degradation shows, in the early months, a greater loss of mass in the leaves from the site of Champenoux. Leaves from the site of Breuil show slower biodegradability, but this difference is transient.

In parallel to these observations, subsampling was performed ( $n = 3$  per treatment) for molecular analyses and enzymatic activity measurements. The monitoring of microbial enzymatic activities showed no major difference between the two litters for the hydrolytic functions (glucosidases, cellobiohydrolases, glucuronidases). On the other hand, concomitantly with the differences in mass loss, we observed an increase in xylosidase activities, which was faster in native litters (Champenoux) than in imported ones (Breuil). Similarly, chitinase activities were significantly higher in native litter (Figure 1).

Interestingly, we also showed that both litter types were colonized by very distinct communities, in terms of composition (Figure 2), although the temporal dynamics of the Ascomycetes / Basidiomycetes distribution was conserved between the two types of litter (Figure 3). Finally, we observed a gradual increase in the ECM species rate in the fungal community in both litters, reaching 30% of fun gal species, at the end of the experiment.

**Main conclusions including key points of discussionn**— Our results show different levels of biodegradation for the two sessile oak litters. Chemical and biochemical analyses have identified certain determinants related to the relative "recalcitrance" of this plant organic matter, in particular the abundance of lignin and certain minerals (Ca, Mg). Moreover, the study of species composition over time (two years) reveals a specific fungal community depending on the origin (and chemistry) of oak litter. Interestingly, after the peaks of hydrolytic activities (carbon mobilization by saprotrophic fungi), we observed a positive correlation between the increase of activities of organic N mobilization (e.g N-acetylglucosaminidases) and the ECM fungi increasing in both litters.

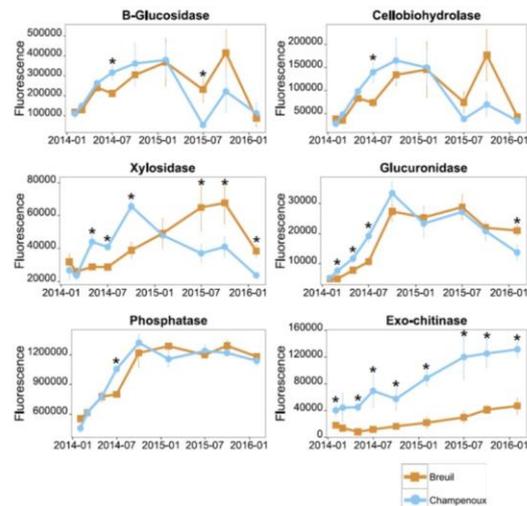


Figure 1. Evolution des activités enzymatiques au cours du temps dans les deux litières

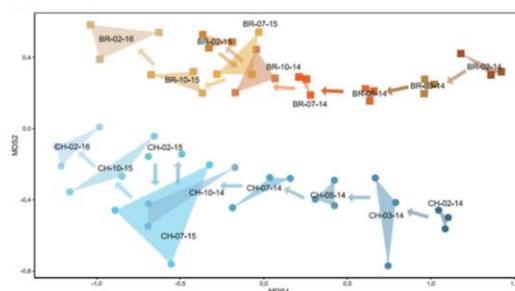


Figure 2. Evolution de la structure des communautés fongiques au cours du temps dans les deux types de litières (NMDS)

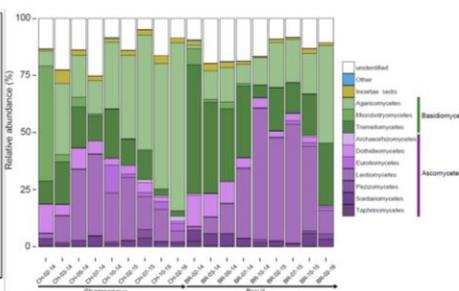


Figure 3. Composition des communautés fongiques (Classes) dans les deux types de litières au cours du temps.

**Future perspectives** — This work demonstrates the functional and temporal complementarity of the saprotrophic and ECM fungal guilds in ecosystem functioning. A cross-approach focusing on litter degradation (on two sites - e.g. Breuil and Champenoux -) could help us to understand the relative role of litter chemistry in comparison to the functional potential of local or non-native fungal communities ("home field advantage") in these decomposition mechanisms. Finally, a future project focused on the expression of fungal and bacterial meta-transcripts (metatranscriptomic approach) would make it possible to identify microbial genetic determinants (eg CAZymes) involved in litter degradation in a perspective of ecological engineering / monitoring for carbon sequestration in forest soils.

### Valorisation —

Maillard F, Ziegler-Devin I, Morin E, Leduc V, Brosse N, Zeller B, Buée M. Transplantation de litières foliaires de chêne : réponses taxonomique et fonctionnelle des communautés fongiques associées à la décomposition. Séminaire de l'Ecole Doctorale RP2E, 2017. (prix du meilleur poster).

Maillard F, Ziegler-Devin I, Morin E, Leduc V, Brosse N, Zeller B, Buée M. Fungal succession on two decomposing leaf litters: interactions and complementary of ecological guilds and their associated functions (Titre provisoire). (article en préparation pour le journal Environmental Microbiology).