

Title: Functional and comparative genomics of pyrophilous fungi

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Project Goals: In this work, we aim to dissect the effects of microbes (fungal and bacterial) on carbon (C) and nitrogen (N) dynamics in post-forest fire soils. Our conceptual framework is rooted in systems biology and ecology, while our experimental approach combines genomics, transcriptomics, metabolomics, microbial community profiling, stable isotope techniques, small scale fire systems (pyrococosms), tightly controlled methods for producing labeled pyrolyzed organic matter, and high-throughput monitoring of C mineralization rates. We have three major research objectives: (1) To determine how dominant post-fire soil microbes affect the fate of PyOM; (2) To assess the interaction between N availability and PyOM mineralization by post-fire microbial communities and individual pyrophilous microbes; (3) To define the network of microbial interactions that facilitate PyOM breakdown over time and the key genes involved in this process.

Abstract text: Understanding post-fire soil systems are essential because they have significant direct and indirect effects on global carbon storage. Fires result in a large amount of carbon that remains resident on the site as dead and partially “pyrolyzed” (i.e., burnt under low oxygen) material with long residency times and constitutes a significant pool in fire-prone ecosystems. Besides, fire-induced hydrophobic soil layers, caused by condensation of pyrolyzed waxes and lipids, increase post-fire erosion and lead to long-term productivity losses. Soil microbes are likely involved in the degradation of all these compounds, yet little is currently known about the organisms or metabolic processes involved. So far, we sequenced and annotated four pyrophilous Basidiomycetes and seven Ascomycetes genomes. In our previous work on Basidiomycetes fungi (Steindorff et al., 2021), we found expansion of genes potentially involved in the degradation of the hydrophobic layer, pyrolyzed organic matter, and mushroom formation. In this work, we focused on the seven ascomycetes genomes and compared them with other 12 non-pyrophilous in the same order and also with 124 genomes at a larger scale, including pyrophilous Basidiomycetes and other organisms with heterogenous lifestyles. Additionally, we explored enriched Pfam domains and CAZymes to identify patterns associated with these organisms’ ‘charcoal-loving’ lifestyle. Our analyses uncovered gene families related to degradation of pyrolyzed organic matter, but these gene families were distinct from those expanded in the pyrophilous fungi in Basidiomycota. . The enrichment analysis revealed families like peritrophin-A, arthropod defensin, aminopeptidases, beta-glucosidase, heat shock proteins,

and fungal fucose-specific lectin. These families might be involved with the pyrophilous fungi' capacity to survive in a toxic environment like post-fire soil. We found a CAZyme CBM14 expanded exclusively in the Pyronemataceae family. This family is mainly found in metazoans, and in fungi, it is only found in some Eurotiomycetes. Since it's a chitin-binding domain, this suggests that secreted CBM14 domain proteins might protect the fungus from microbial attacks in its soil habitat. Another interesting finding is that pyrophilous fungi have larger proteins than non-pyrophilous, being in an intermediate state to thermophiles. Pyrophilous fungi are commonly found fruiting after fire events, therefore, passing through their sexual stages in this process. To make an in-depth comparison of these conditions, we analyzed the available transcriptomic data of *Pyronema domesticum* grown in charcoal and during sexual development. We performed a co-expression network analysis and found two modules with the most differentially expressed genes in charcoal and sexual development. Gene Ontology categories like chitin/carbohydrate/lipid/superoxide metabolism and transport were found in both modules, showing that such processes are likely required to grow in the presence of charcoal and sexual development. The transcription factor STE12, known to be involved in sexual development in yeast and filamentous ascomycetes, was up-regulated in both conditions when compared with their respective controls. This study will improve our understanding of this unique lifestyle of pyrophilous fungi and their role in post-fire carbon cycling.

References/Publications

Steindorff AS, Carver A, Calhoun S, Stillman K, Liu H, Lipzen A, He G, Yan M, Pangilinan J, LaButti K, Ng V, Bruns TD, Grigoriev IV. Comparative genomics of pyrophilous fungi reveals a link between fire events and developmental genes. *Environmental Microbiology*. 2021 Jan;23(1):99-109.

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