

**Title:** A mechanistic approach to parsing pyrophilous lifestyles of fungal and bacterial isolates

**Authors:** Monika Fischer<sup>1\*</sup> (monikaf@berkeley.edu), Timothy Berry<sup>2\*</sup> (tdberry@wisc.edu), Andrei Steindorff<sup>3\*</sup> (assteindorff@lbl.gov), Frances Stark,<sup>1</sup> Nayela Zeba,<sup>2</sup> Igor Grigoriev,<sup>3</sup> **Thea Whitman**,<sup>2</sup> Matthew Traxler<sup>1</sup>

**Institutions:** <sup>1</sup>University of California-Berkeley, Berkeley, CA; <sup>2</sup>University of Wisconsin-Madison, Madison, WI; and <sup>3</sup>Lawrence Berkeley National Laboratory, Berkeley, CA

**Website URL:** <https://whitmanlab.soils.wisc.edu/research/>    <https://traxlerlab.berkeley.edu/>

**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 (pyrocosms), tightly controlled methods for producing labeled pyrogenic organic matter (PyOM), 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:** Wildfires are a fundamental ecological process with dramatic effects on ecosystem function and nutrient cycling (including carbon). Soil fungi and bacteria play a key role in post-fire cycling of pyrogenic organic matter, in addition to their roles in other biogeochemical cycles. We are taking a multifaceted approach to investigating the response of specific organisms to wildfire and pyrogenic organic matter additions, using comparative genomics and transcriptomics on isolates that are known pyrophiles or that can grow with PyOM as their only C source. This research has included sequencing the genomes of pyrophilous fungal taxa, including *Lyophyllum atratum*, *Coprinellus angulatus*, *Pholiota molesta*, *Crassisporium funariophilum*, *Pyronema domesticum*, *Pyronema omphalodes*, *Tricharina praecox*, *Geopyxis carbonaria*, *Morchella snyderi*, and *Peziza echinospora*. Comparative genomics have indicated that the genomes of pyrophilous fungi may be enriched in or have expansion of carbohydrate-active enzymes (CAZymes), families associated with stress response and the initiation of fruiting bodies, both of which may be relevant in the post-fire environment.

Fungi in the genus *Pyronema* dramatically dominated the soil fungal community within weeks to months after the 2013 Rim Fire around Yosemite National Park (CA, USA). *Pyronema domesticum* isolated from this fire was used to identify how it survives and interacts with the cycling of carbon or pyrolyzed carbon. Leveraging the recently sequenced genome, we did an RNAseq experiment with *P. domesticum* grown on four different carbon sources; 750°C PyOM, wildfire burned soil, sucrose minimal medium, and water (no carbon). The PyOM induced

expression of a comprehensive set of genes required for the breakdown and metabolism of aromatic and polyaromatic compounds, which are characteristic of PyOM. We further confirmed that *P. domesticum* could mineralize  $^{13}\text{C}$ -labelled 750°C PyOM to  $\text{CO}_2$ . Taken together, our results show that the pioneering post-fire fungus *P. domesticum* can liberate carbon from complex PyOM and return it to the carbon cycle.

In addition to the fungal isolates described above, over 70 bacterial isolates have been collected from fire-affected soils and assayed for their ability to mineralize PyOM. The 12 isolates with the most robust growth on PyOM agar were cultured on a medium containing  $^{13}\text{C}$ -enriched maple wood PyOM. The isotopic composition of the  $\text{CO}_2$  produced by each of these isolates conclusively demonstrates the mineralization of PyOM. A similar approach has been taken to assay the potential of cultured isolates to degrade specific molecular components found in PyOM extracts (*i.e.* polyaromatic hydrocarbons and methylphenols). After sequencing the genomes of these 12 isolates, we aim to use isotope-enabled respiration studies to determine the primary pathways necessary for bacterial PyOM mineralization.

Together, these findings illustrate decisive steps toward identifying the microbial taxa involved in post-fire PyOM degradation and the molecular mechanisms they use to do so.

### References/Publications

1. Fischer, M.S., F.G. Stark, T.D. Berry, N. Zeba, T. Whitman, and M.F. Traxler. (2021). Pyrolyzed substrates induce aromatic compound metabolism in the post-fire fungus, *Pyronema domesticum*. *Frontiers in Microbiology*, 12, 729289.
2. Berry, T.D., C. Creelman, N. Nickerson, A. Enders and T. Whitman. (2021). An open-source, automated, gas sampling peripheral for laboratory incubation experiments. *HardwareX*, 10, e00208.
3. Steindorff, A.S., A. Carver, S. Calhoun, K. Stillman, H. Liu, A. Lipzen, G. He, M. Yan, J. Pangilinan, K. LaButti, V. Ng, T.D. Bruns, and I.V. Grigoriev. (2021). Comparative genomics of pyrophilous fungi reveals a link between fire events and developmental genes. *Environmental Microbiology*, 23, 99-109.

**Funding Statement:** *This work was funded by the Department of Energy, Systems Biology Enabled Research on the Roles of Microbiomes in Nutrient Cycling Processes program, grant DE-SC0020351 to Thea Whitman, Thomas D. Bruns, Matthew Traxler, and Igor Grigoriev. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231.*