**Microbial Responses to Pyrogenic Organic Matter: Cultivating Isolates, Ageing PyOM, Tracing Gas Fluxes, and Meta-analysis**

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**Project Goals:** The frequency of large, high severity wild fires is increasing in the western US and in regions around the world due to long-term fire suppression strategies and climate change [1]. These fires have direct, negative effects on soil carbon stocks through combustion, but they have indirect and potentially positive effects on soil carbon stocks through the production of pyrogenic organic matter (PyOM) that has a long residence time and constitutes a major pool of C in fire-prone ecosystems [2-4]. Soil microbes are likely to be involved with the degradation of all these compounds, yet little is currently known about the organisms or metabolic processes involved. **We are dissecting the effects of microbes on post-fire soil carbon dynamics by using a systems biology approach that couples small experimental "pyrocosms", highly controlled production of $^{13}$C-labeled pyrolyzed substrates, genomics, transcriptomics, stable isotope techniques, and mass spectrometry to address the following objectives:** (1) Develop improved genomic and other -omic resources for the dominant microbes of fire-affected soils; (2) Determine the temporal response of soil microbes to fire and to PyOM additions; (3) Characterize the temporal patterns of degradation of different sub-fractions of PyOM.

The fungal community in fire-affected soils is increasingly well characterized, and the broader team continues to develop genomic, proteomic, and metabolomic resources for the study of these organisms. In contrast, the bacterial community in fire-affected soils is less well understood and in need of further study. To help meet this need we have isolated >80 bacterial strains from fire-affected soil with the potential to mineralize PyOM. Sequencing of the 16S rRNA genes have identified the majority of theses strains as being members of the *Actinobacteria* phylum, with *Streptomyces* and *Pseudonocardia* being the most prominent.

A custom gas sampling device, the multiplexer, has been constructed to facilitate the analysis of PyOM mineralization by bacterial isolates and whole soils during incubations with $^{13}$C enriched PyOM. In conjunction with a Picarro cavity ringdown spectrometer, the multiplexer allows for rapid measurement of the $CO_2$ concentration and isotopic composition in the headspace of up to 56 incubation vessels. We present data from a small-scale soil incubation using $^{13}$C enriched sugar maple PyOM to demonstrate the utility of the instrument. While typical incubations using an isotope ratio mass spectrometer might achieve one measurement per day, we sample the headspace of each soil incubation 11 times over 36 hours; this frequent sampling allows for the detection of fine-scale $CO_2$ flux dynamics obscured by other methods. Additionally, the large sample number accommodated by the multiplexer allows for the simultaneous inclusion several treatments with replication in a single experiment.

Our understanding of the chemical and biological stability of PyOM has been revised in recent years with studies observing a change in the physico-chemical properties of PyOM when it is deposited in soil [5]. These changes can occur after PyOM has persisted in soil for a very long time and the process is often referred to as 'ageing' of PyOM [6]. PyOM ageing is likely to influence the degradation of PyOM materials by soil microbes, consequently affecting native soil carbon stocks. We present data from an experiment where we compared pyrogenic carbon mineralization between aged and unaged PyOM by *Streptomyces sp.,* isolated from fire affected soils. We aged eastern white pine wood PyOM produced at 350 and 550 °C using a combination of physical and chemical treatments in lab to accelerate the process of ageing. We incubated aged and unaged pine wood PyOM with *Streptomyces sp.* for a period of 14 days during which the pyrogenic carbon mineralized was measured by sampling the head space of each incubation every 48 hours over a period of 14 days using the Picarro-multiplexer setup. We observed that both chemical and physical ageing reduced pyrogenic carbon mineralization compared to unaged PyOM in the case of 350 °C PyOM. However, in the case of 550 °C PyOM, we observed higher pyrogenic carbon mineralization for the chemically aged PyOM compared to both physically and unaged PyOM incubations.

Finally, we performed a meta-analysis of the effect of PyOM additions on soil bacterial community composition, with the goal of determining: (1) Is there a detectable and consistent "charosphere" community that characterizes PyOM-amended soils – *i.e.*, which is more important for determining bacterial community composition, soil properties or PyOM additions? (2) Are there consistent responses at the phylum level to PyOM amendments? (3) Can we identify individual PyOM-responsive taxa that increase in relative abundance consistently across different soil types? We did not observe consistent community or phylum-level responses to PyOM amendments across studies. However, we did identify genera that tend to be enriched with PyOM additions, including *Sphingomonas sp., Nocardioides sp.*, and *Mesorhizobium sp.*, which may be putative PyOM-degraders.

**References**


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