Experimental pyrocosms demonstrate key features of the autecology of post-fire fungi.

Thomas D. Bruns (pogon@berkeley.edu) [1], Akiko Carver[1], Kyra Stillman[1], Catharine A. Adams[1], **Thea Whitman**[2]
[1]Dept. Plant and Microbial Biology University of California, Berkeley, [2] Department of Soil Science,University of Wisconsin, Madison

Project Goals: Pyrophilous fungi fruit prolifically after fire and are generally restricted to post-fire environments (Peterson 1970, Hughes et al 2020). To increase our understanding of the behavior of these fungi we have used experimental units termed "pyrocosms" to control soil heating and incubation conditions. Here we used soils from multiple forest, meadow, and agricultural sites to test the generality of the *Pyronema* response and we varied incubation conditions to see if other pyrophilous fungi could be stimulated. These manipulations were designed to test the following four hypotheses:

H 1 *Pyronema* inoculum is widespread in forest soils;
H 2 *Pyronema* inoculum is more abundant in forest soils than adjacent meadow soils;
H 3 more genera of pyrophilous fungi will develop with longer incubation times;
H 4 prolonged chilling of soil to simulate winter will stimulate additional pyrophilous fungi.

Background:
To understand the interactions of pyrophilous fungi with post-fire soil carbon we previously isolated a set of common pyrophilous fungi, sequenced them, and assembled their genomes (Steindorff et al 2021, Steindorff et al. poster– this conference), and we are studying their gene expression under controlled conditions (Whitman et al. talk – this conference). However, the factors that allow these fungi to dominant post-fire soils is poorly known. Here we used pyrocosms to subject soil samples to experimental fire in which soil heating and fuel composition was held constant, while incubation conditions involved either ambient temperatures or a simulated winter (vernalization) followed by 32 weeks of incubation. Development of fungal communities was monitored by sampling at 7 time points, followed by high-throughput sequencing of the ITS region. The results were used to test the four hypotheses listed in the goals.

Results
1) *Pyronema domesticum* was found to be widespread in conifer forest and adjacent meadow soils and responded rapidly to burning. *Pyronema* appears to be more abundant in forest compared to meadow soils. In Sierra and Cascade soil samples it accounted for over 70% of all sequences one to two weeks after burning , while in the central California coastal forest sample it accounted for a lesser, but still impressive 20% .
2) Known pyrophilous fungi that developed in some units were: *Pyronema spp*. (3 OTUs), *Pholiota aff highlandensis* (1), *Geopyxis spp.* (2), *Lyophyllum aff anthracophila* (1), *Neurospora aff. tericola* (9); however, with the exception of *Pyronema* and *Lyophyllum*, sequence abundances were < 1% at all sample times.
3) Some taxa not known to be pyrophilous responded positively to fire.
4) *Lyophyllum aff. anthracophila*, a known pyrophilous fungus, responded strongly and positively to vernalization of soil post-fire after 16 weeks of incubation and was restricted to forest soils .

Conclusions: Hypothesis (H_1) is based on the assumption that pyrophilous fungi reside in these soils between fire events as spores or sclerotia. Our results now support this hypothesis, as the rapid, predictable response of *Pyronema* is consistent with a stimulation of resident inoculum. Dispersal seems unlikely to explain the pattern, since the species rarely fruits in unburned habitats and the response was too rapid, consistent, and sample-specific to be accounted for by rare dispersal events. **Hypothesis (H_2)** that pyrophilous fungi, such as *Pyronema spp*., would be more abundant in forest vs meadow soils is based on the idea that higher course-fuel load in forests create hotter temperatures at greater depths, which have created larger inoculum pools. Our results with both *Lyophyllum* and *Pyronema* support this view. Our initial pyrocosm study resulted in only a strong response by *Pyronema spp.* (Bruns et al 2020). We predicted that longer **incubation time (H_3)** and the **simulated winter (H_4)** would result in growth of additional known pyrophilous fungi. The response of *Lyophyllum aff anthracophila* to both these factors demonstrated the validity of these hypotheses, but in a rather restricted way, as other known pyrophilous fungi, although present, did not exhibit the large increases seen with *Pyronema* or *Lyophyllum*. However, several unknown fungi responded in ways that suggest they may be unrecognized pyrophilous fungi. Our results show that additional pyrophilous fungi are present in the samples, and we are optimistic that further alteration of simple factors in the pyrocosm

environment will stimulate abundant growth of some of these additional pyrophilous fungi. Overall, the post-fire soil environment is relatively simple and likely to be highly predictable at multiple scales. Variation in fuel loads within a forest are predicted to create undulating depths of thermo-chemically defined zones at the landscape scale (Bruns et al 2020). Timing and severity of fires, though variable, are predictable in aggregate by the fire regimes associated with the plant community type and climate. Correlation between these known variables and autecology of pyrophilous fungi is highly likely, and the pyrocosm approach provides a tool to dissect their niche spaces.

References

Hughes, KW et al. 2020. **Mycologia** 112: 677-698

Bruns TD et al. 2020. **PLOS ONE** 15(3):e0222691. https://doi.org/10.1371/journal.pone.0222691

Petersen PM. Danish Fireplace Fungi. **Dansk Botanisk Arkiv.** 1970;27(3):1-97

Steindorff, AS et al. 2021. Environ Microbiol, 23: 99-109. https://doi.org/10.1111/1462-2920.15273

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