

## Effects of biochar addition on fungal communities: Implications for forest insects

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### Abstract

Many western forests are severely overstocked, creating a critical need to understand how forests can be thinned while maintaining biodiversity and other ecosystem services, such as carbon sequestration. Wood-inhabiting fungi play a critical role in carbon cycling and carbon

sequestration. However, the effects of different management techniques on wood-inhabiting fungal communities have rarely been investigated. Here, we implemented a manipulative experimental design to determine the effects of thinning on fungal communities, with four levels of slash retention as well as four combinations of compensatory soil amendments (non-amended control, biochar, fertilizer, or fertilizer+biochar). We sampled our treatment plots for three years using above- and below-ground wood stakes (pine and aspen) and also conducted annual surveys of woody material for fungal fruiting bodies. Fruiting bodies were identified using a combination of morphology and DNA sequencing, while high-throughput DNA sequencing was used to identify fungi that colonized the wood stakes. Although differences in diversity and richness of fruiting bodies were not observed across treatments, the different levels of slash retention did affect the community composition of wood-inhabiting fungi observed as fruiting bodies. In contrast, the DNA-based survey of wood-colonizing fungi indicated that fungal communities were sensitive to levels of slash retention as well as different soil amendments, including biochar addition. Future work will determine how these changes in the fungal community may affect decomposition rates and explore how changes in the fungal community may affect other organismal groups, such as the many forest insects that interact with fungi.

### **Research goals and study design**

Our research goals were to contribute to the understanding of how wood-inhabiting fungal communities respond to different forest management practices in western North America, while specifically investigating how richness, diversity and community composition of fungi may change in relation to different levels of slash retention and to the addition of different combinations of soil amendments (fertilizer and biochar) (Draeger 2018). Two sites were installed on the University of Idaho Experimental Forest in 2013 with the sites being separated by approximately 1 km. Sixteen 400 m<sup>2</sup> study plots were established at each site with biomass treatments at four levels (no thinning control, no slash removed, slash left on site, or 2x the amount of slash left on site) and four types of soil amendments (non-amended control, biochar, fertilizer, or fertilizer+biochar). Biochar was from Evergreen Forest Products (Tamarack, ID, USA) and was produced from mixed-conifer mill residue at 890°C (Sherman et al. 2017) and applied hydraulically to each plot at a rate of ~2.5 Mg ha<sup>-1</sup>. This created 16 different treatment combinations (4 slash retention levels by four types of soil amendment) replicated across two sites.

### **Surveys of wood-inhabiting fungal fruiting bodies**

Fungal fruiting body sampling methods follow established protocols (Mueller et al. 2004). The following characteristics were reported when a fruiting body was encountered: preliminary fungal species identification, substrate type (branch, log, suspended log, snag, stump, and living tree), wood substrate species, diameter class, and decay class following the five-class system of Maser et al. (1979). Fruiting bodies were inventoried unless their state of degradation precluded identification.

Over 3 years of survey, 1,002 wood-inhabiting fungal fruiting bodies were collected and identified using morphology and DNA-sequencing. One hundred and twenty-nine species were

identified in total, all of which were in the phylum Basidiomycota. Many species (22 of the 129 species, or ~17%) did not match known species descriptions, and species accumulation curves indicate that more species would be found with more extensive sampling. No significant differences were observed among treatments for species richness or diversity. However, fungal community composition was influenced by biomass retention, with the level of slash retention significantly influencing the fungal community composition in the first and third years of sampling ( $p < 0.04$ ). Soil amendments generally did not affect the fungal community, although there was a moderate influence on the fungal community composition in the second year of sampling (2015,  $p = 0.064$ ), with fertilization being the primary driver of differences ( $p < 0.02$ ). Biochar amended plots did have the greatest species richness of fruiting bodies, although the difference was not statistically significant.

### **Surveys of wood-inhabiting fungi using high-throughput DNA sequencing**

Using protocols described by Jurgensen et al. (2006), 25 loblolly pine (*Pinus taeda*) and 25 aspen (*Populus tremuloides*) stakes (2.5 x 2.5 x 30 cm) were inserted vertically to a depth of 30 cm in the mineral soil of all treatment plots. An additional 25 pine and 25 aspen stakes (2.5 x 2.5 x 15 cm) were affixed on top of the surface organic horizons. Installation occurred in spring of 2014. Sampling occurred each year for three years, and during sampling, five aspen and five pine stakes were removed from both the mineral soil and litter layer location of each plot and wood shavings were extracted from each stake using DNA-sterile methods.

Fungi inhabiting the wood stakes were identified using high-throughput amplicon sequencing (HTS). Samples for DNA sequencing were collected using methods similar to those described by Lindner et al. (2011). HTS was performed on the fungal ITS2 fragment using an Ion Torrent PGM and bioinformatic analyses were performed using AMPtk (Palmer et al. 2018). A single-copy positive community control (SynMock) was used to parameterize the bioinformatic pipeline. We used multivariate hypothesis tests to compare differences in fungal community composition across treatments and sampling methods.

After three years of sampling, over 2,000 operational taxonomic units (OTUs) were identified. Approximately 50% of identified taxa were assigned to the phylum Ascomycota and 33% to Basidiomycota. Species of stake and location (surface or within the mineral layer) were highly significant in influencing both fungal richness and fungal community composition ( $p < 0.01$ ). Biomass treatments significantly influenced the fungal communities present within surface stakes every year of data collection ( $p < 0.05$ ), whereas for the stakes in the mineral soil, biomass treatment was only significant in the second year ( $p < 0.05$ ). Initially the 2X biomass treatment had fewer OTUs, although by the third year the 2X biomass treatment had the greatest OTU richness. Fungal community compositions was also significantly influenced by soil amendments. Fungal colonization of both aspen and pine surface stakes was influenced by soil amendments in the first year ( $p < 0.01$ ), whereas only surface aspen and mineral pine stakes were influenced by soil amendments in the second year ( $p < 0.05$ ). By the third year, both pine and aspen surface stakes had significant differences in fungal community composition due to soil amendments ( $p < 0.05$ ).

### **Comparison of methods**

The two sampling methods revealed significantly different fungal communities. Fruiting body surveys sampled a range of substrates and identified Basidiomycota species, including multiple important forest pathogens. DNA-based methods produced more data, identified more OTUs ( $p < 0.001$ ) and were more sensitive to biomass removal and soil amendment treatments. Both methods have advantages and disadvantages, and success with each method depends on the questions being asked and the resources available.

### **Conclusions and future directions**

Biomass harvesting and soil amendments have the potential to alter fungal communities in conifer-dominated forests of western North America and this has implications for forest health and productivity. Changes in the fungal community may affect multiple ecosystem services, such as humus formation and carbon sequestration. In addition, the changes in the fungal community may have cascading effects on other organismal groups that interact with fungi, especially for insect groups that interact closely with decomposing wood, such as beetles and termites. More research is needed to understand the interactions among forest management techniques, wood-decomposing fungal communities, and wood-associated insect groups.

**Keywords:** biochar, decay, decomposition, wood-decay fungi

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