Fungal Degradation of the Woody By-products of Forest Management Activities
First Year Report, 2015

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Introduction

Over a century of fire suppression in Western American forests has led to a dangerous situation: Trees are over populated and exist in non-historic configurations. Many forests are badly stressed by extreme competition for water and nutrients. Land managers have had to implement forest mitigation techniques to deal with these problems. Large quantities of wood chips may be left on the forest floor by several types of mitigation treatments including mastication, hydro-axing, chipping . While economically necessary, this material can negatively impact the local ecosystem. The cost of removing timber for non-commercial use is expensive, and sometimes logistically impossible. Many consider these techniques as merely "re-arranging" fuels by converting Arial timber to a horizontal layer on the forest floor. Overly thick deposits can take decades to degrade naturally, and can have a severe impact on the forest understory.

- *The use of native fungal species may be able to breakdown this material, speeding the recovery of the forest floor and kick-starting a return to natural systems that have been disrupted by these management practices. As the primary saprophytes consume their preferred portion of the substrate (high energy compounds), the secondary and tertiary decomposers will then move in to further reduce the material to humus and as a by-product lower the energy content of the substrate.*

This study is being conducted to see if techniques of myco-remediation offer promise as a tool for forest management practitioners by allowing for the post treatment of these woody byproducts. We sought to initially test the efficacy of using native mushroom species to digest wood chips and document the procedures and their results. Our hope is to create baseline guide for these types of treatments and to document the rates of inoculation, rates of spread, and efficiency of degradation over time.

Wood chips are composed mainly of cellulose and lignin, two extremely tough compounds. Cellulose is a linear chained polysaccharide, and lignin is a cross linked phenol polymer which

glues the fibers of cellulose together. Various types of fungi are capable of synthesizing enzymes that can break the carbon hydrogen bonds of these compounds. In their most basic forms, white rotters and brown rotters are capable of digesting lignin and cellulose respectively. Mushrooms, with their production of large fruiting bodies and the attendant nutrient and energy demands of their growth, offered the potential to degrade large quantities of material with each fruiting (flush).

- There are four basic types of fungi: Endophytic, Saprophytic, Parasitic, and Mycorhyzial. Saprophytes are primary decomposers and live on dead materials~~,~~ such as compost, excrement, and wood. We chose to work with wild collected wood decomposers as the starting point in our efforts to "train" a mushroom to prefer wood chips from our test site. Their normal habitat would exist on dead standing or downed logs of various species. Man-made wood chips are not a normal food source for any of these species. The enzymatic "machinery" of these species produces extracellular carbohydrate active enzymes (CAZ) in a "suite" with reactive oxygen species (ROS) while decomposing lignin, hemi-cellulose and cellulose. This enzymatic activity can inhibit bacterial growth on the substrate.

We began with 5 candidate species: Pleurotus pulmonarius (Oyster), Stropharia rugosoannulata (King Stropharia), Agaricus silvicola (Sylvan Agaricus), Lycoperdon perlatum (Gem-studded Puffball) and Hypsizygus tessulatus (Box Elder Oyster). We later added Morchella angusticeps (Black Morel). All specimens had been collected by James Weiser at vicinities around central Colorado.

1. Training

Mycoremediation is the use of fungi to aid in various types of environmental restoration. Mycodegradation is the use of fungi to decompose various substrates, in our case: wood chips. The technique of fungal "training" involves a sort of accelerated selection where the natural variability within species is used to generate a new strain suited to a specific purpose. Harbhagan Singh is the pioneer in the training of fungi for a wide variety of applications. Putnam and Gates have been using a similar training system to select for coral polyps that are better adapted to changing ocean conditions in Hawaii. Paul Stamets practices and promotes these techniques actively.

Specimens were obtained by either tissue/mycelia culture or spores and inoculated on Agar dishes prepared with powdered wood chips (see Appendix A). On Dec. 17th 2014, the cultures were incubated and checked daily. Spoiled cultures were immediately discarded. On March 17th 2015, we discovered that the species that thrived in dish culture were S. rugos~~s~~o-annulata, P. pulmonarius, and M. angusticeps. These were chosen for "grow out". They were transferred to

quart jar cultures of approximately 80% wood chips & 20% millet grain, with water and gypsum buffering. No other nutrients were provided. Total infestation of the medium took between 3-5 weeks.

On April 27th 2015, the spawn from the jars were used to inoculate air-flow spawn bags that would be used in the field for introduction to the wild. The medium was 100% wood chip and water: the mass was approximately 8lbs/bag. The Stropharia and Pleurotus enveloped their media in 2-3 weeks. The Morchella took about 6 weeks. Once the spawn was complete they were moved to a dark cool area to await sowing at the test site.

2. Test Site

The test took place on Denver Mountain Parks (DMP) property in Jefferson county, Colorado. Two species were chosen by the DMP Forester and Naturalist. Two separate sites were chosen approximately 100 yards apart to avoid any cross contamination of species. Five- 1m ^{2} beds were established within each site for the test. Four plots were inoculated with spawn while the fifth was left as a control.

Test Bed Layout:

Bed 0	Pleurotus pulmonarius *Solo*	Morchella angusticeps *Solo*	Bed 5
Bed 1	*P. pulmanarius w/ Jute matting*	*M. angsticeps w/ Jute matting*	*Bed 6*
Bed 2	P. pulmanarius w/ nutrients (fish emulsion, humic acid preparation)	M. angusticeps w/nutrients (fish emulsion, humic acid preparation)	Bed 7
Bed 3	P. pulmanarius w/ nutrients & Jute matting	M. angusticeps w/nutrients & Jute matting	Bed 8
Bed 4	CONTROL	CONTROL	Bed 9

Jute matting was used to access any benefit from covering; such as shading and moisture retention. Nutrients were standard nursery preparations of fish emulsion and humic acid. One gallon of each solution was mixed in with the woodchips during bed preparation. The beds were constructed to a standard depth of 12”.

3. Inoculation

On June 12th 2015, one bag of spawn was buried in each plot, near center at a depth of 9” to 10”. Tools were sanitized before this procedure. Gloves and masks were used when (briefly) handling the spawn to reduce the risk of contamination. All equipment was re-sanitized and new gloves/masks were used from site to site to eliminate cross-contamination. Matting was placed where appropriate and the site vacated. We would not allow any artificial watering or

nutrient addition after the initial set.
A screen test was used to measure the initial resistance of the chips to simple erosion. A ¼" mesh screen was used to abrade the chips for a timed interval of 2 minutes. The amount passed was weighed and compared to the sample weight. The percentage of mass excluded to mass allowed formed our substantive measurement.

Initial conditions:
Ambient temp: 70 °F, humidity 80%, rain/sun, soil temperature: 57 °F
Average bed density 280 l, 111.72 kg, (32.7 kg/l)
Chip moisture: Pleurotus bed: 70.4%, Morchella bed: 68.9%
Rate of inoculation (by weight): 1/35.7 (spawn/wood chips)
Screen test- 2litre (798 gr) 142.7gr passed ¼" screen in 2 min. = 17.7% (avg. 2 tests)

4. Monitoring
The beds were checked 6 times over 5 months. At each visit a visual check was made for fruiting bodies. Bed depth and temperature was measured, as were the ambient weather conditions. To minimize intrusion, a random number chart was used to select 2 of the 5 beds at each site to measure each visit. Chip samples were taken to measure moisture contentent. If fruit was present, it was harvested and weighed. The samples were kept as a future spore source.

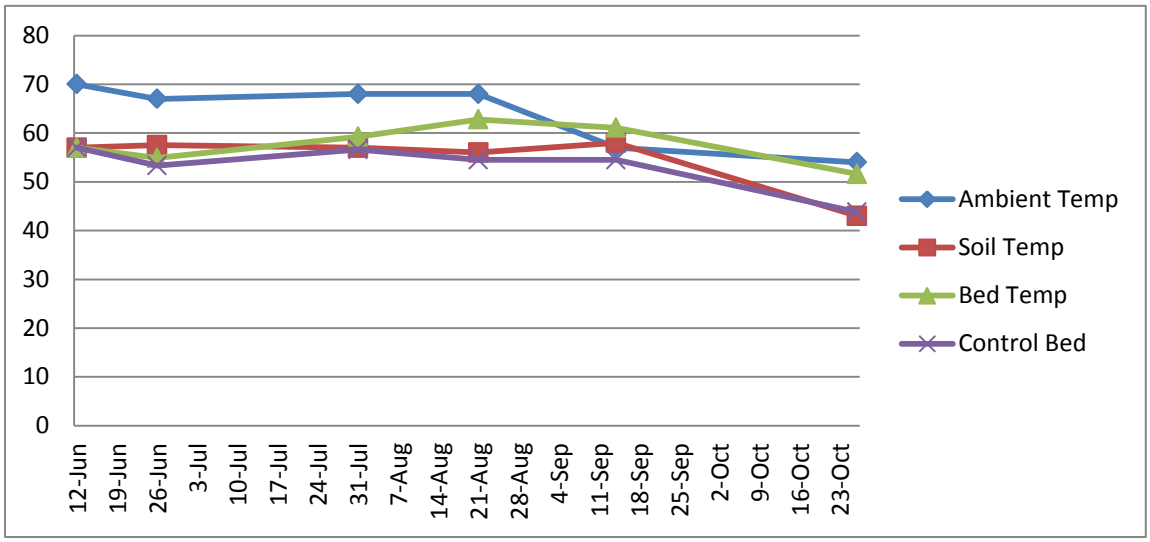
The final monitoring was conducted on October 26th 2015. At this time final measurements have been made and samples taken for an end of season fruit collection and final screen test. The random chart was also used to select one bed for disassembly in order to measure the extent of mycelial spread. The bed was excavated with trowels, one inch at a time beneath a grid to ultimately measure the depth and extent of infiltration.

No fruit was detected on the M. angusticeps site at any time. Evidence of either deer or bear vandalism was noted on the July 31st visit. All data is for the P. pulmonarius site only. Monitoring of both sites will continue in the following years and be reported annually.

5. Results
This test had a 100% productivity rate on the P. pulmonarius site. Each bed fruited twice with 75% of beds fruiting 3 times.

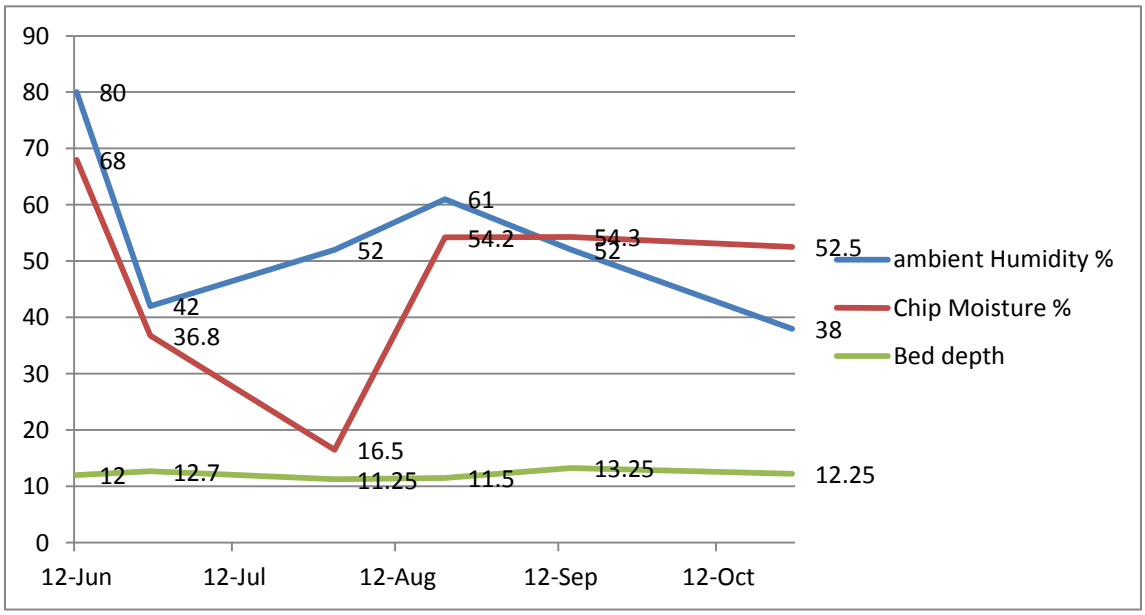
Raw Data:

Table 1: Monitoring Temperature Data



Soil temperature remains fairly constant, as one would expect, becoming cooler after September when the region had begun a cool down. Bed temperature seems to always be slightly higher than soil temperature and increases significantly after the initial fruiting (July) and remains higher than soil temp. throughout the rest of the test. These are not the extreme temps one would expect from microbial decomposition (composting), which is a very noticeable indication of the mushrooms activity. Any increase in bed temperature would be expected to favor the activity of secondary decomposers as well.

Table 2: Monitoring Moisture/ Bed Depth Data



Chip moisture fluctuates greatly during the beginning of the season, leveling out between the first and second flushes. This may be due to the mushrooms themselves exerting a moisture buffering effect on the chips. More study, and particularly more sample points will be needed to fully understand this result. The bed depth seems to rise and fall out of sequence with either the chip moisture content or the ambient humidity. An interesting result is that for beds monitored while flushing, the bed depth tended to be lower than baseline depth of 12", with times between flushes showing a greater substrate depth. Again, more data will be needed to understand these phenomena.

Table 3 Rain Accumulation

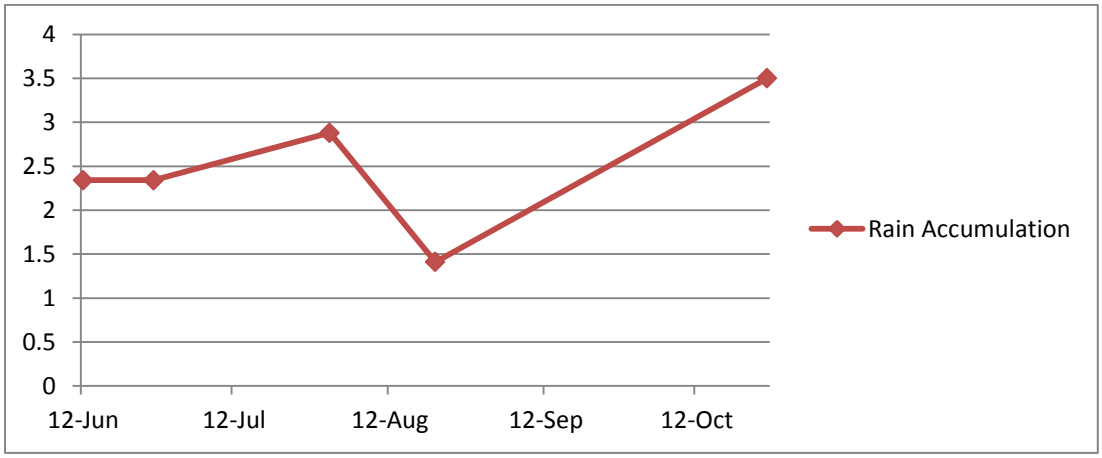
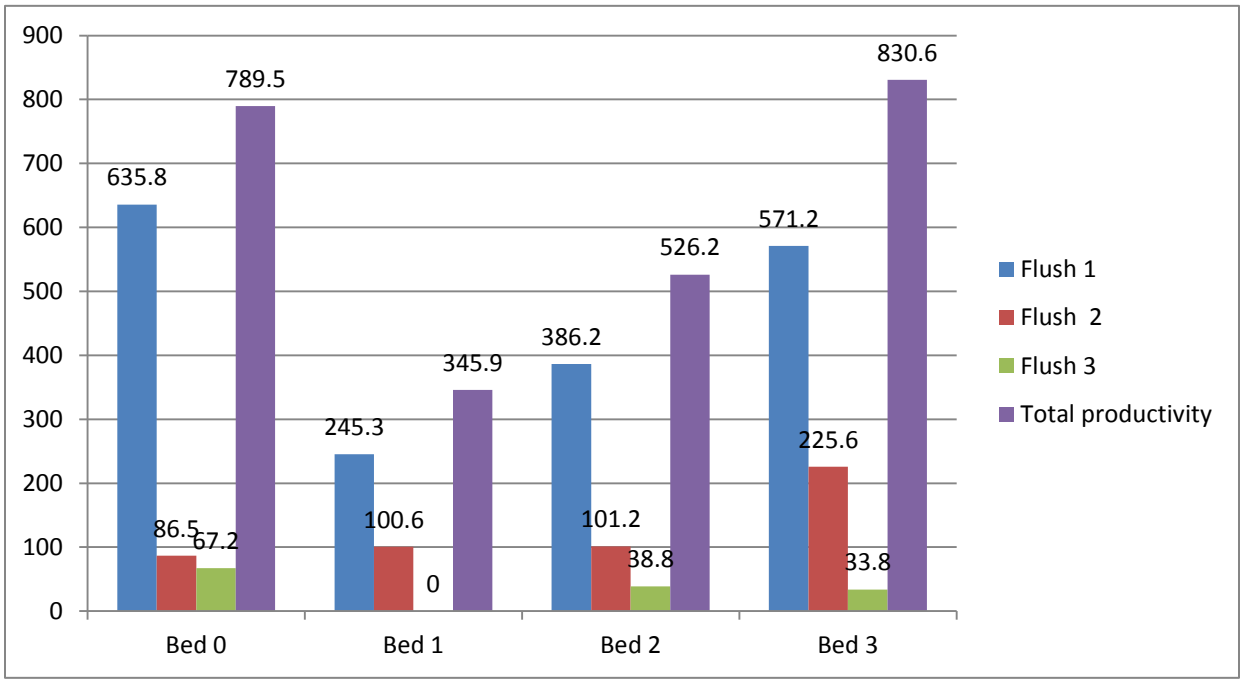
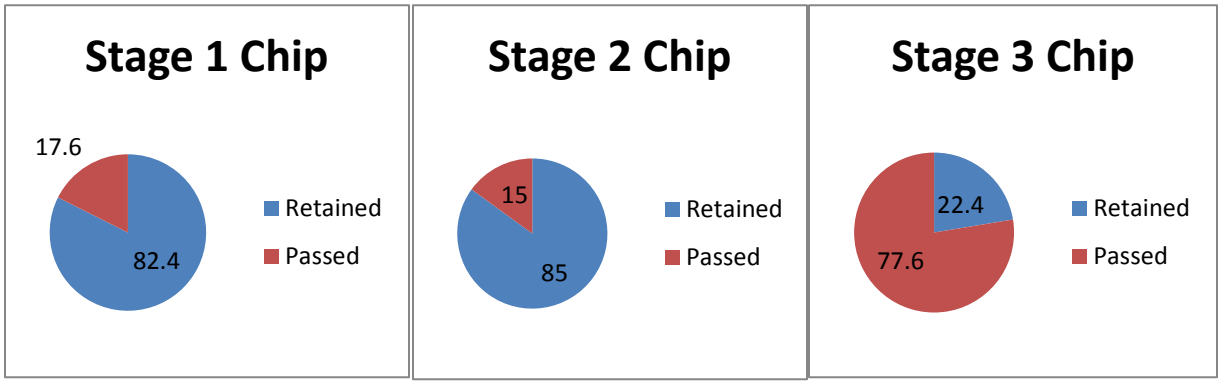


Table 4: Fruiting Data (in grams, wet weight)



Roughly 2.5 kilograms of mushrooms were produced by the mushrooms in this test during season 1. The most productive beds were the solo beds (no amendments or covering) and the covered and amended beds. Monitoring was randomly practiced and a check of numbers of samples taken from each bed are relatively equal (Bed 0- twice, Bed 1 - twice, Bed 2- 3 times, Bed 3- 3 times) so that any effect from under or over sampling is ruled out. Hopefully the next few seasons of monitoring will shed more light on the issue of amendments, but as of this sample collection it seems that these particular organisms are without preference re: shade and/or nutrients.

Table 5: Chip Degradation

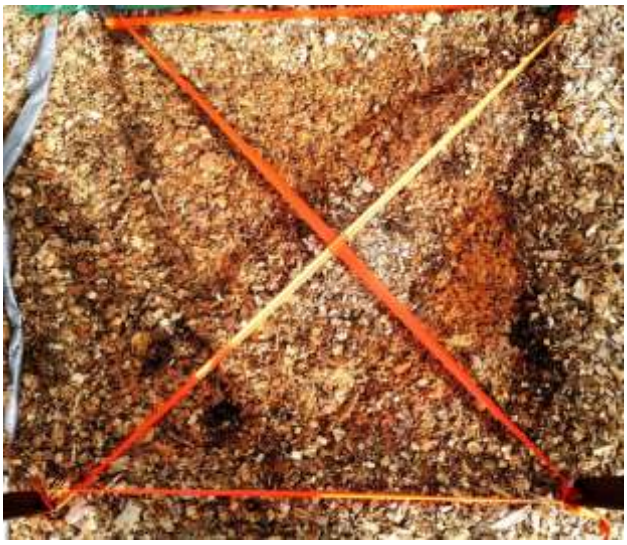


The main test used for chip degradation was the screen test. A standard volume of chips were vigorously pressed and rubbed against a ¼" screen for a set interval of 2 minutes. While volume was constant, each test run was weighed before and after testing. Results are presented as percentages by weight of the amount retained or passed through the screen. The chip degradation is extremely interesting. During the course of this study it was discovered that there are three distinct stages that the wood chips go through.

Stage 1 - Raw chips. Stage 1 chips have the solidity of chunks of wood and are reluctant to pass through the ¼" screen. *2 litres by volume passed 17.6% through the screen in 2 minutes.*

Stage 2 Are fully engulfed, but not yet digested chips. They vary from raw in two noticeable ways. They are smaller, and they are deep yellow red (see fig 3). They are actually more reluctant to break apart on the screen than raw chips. We conjecture that this, like the darker color, is the result of pre-digestion leaving a shell of lignin around the shrinking chip. More research is needed to understand this. *2 litres by volume passed 15% through the screen in 2 minutes.*

Stage 3 Chips are hardly recognizable as wood chips, having been more or less completely digested. Stage 3 occurs within the mycelia involved in fruiting. Stage 3 appears as a mass of mycelia and compost. There is a near complete reversal of what does and does not pass the screen. The retained mass is spongy and dark(see fig 4). *2 litres by volume passed 77.6% through the screen in 2 minutes.*



The bed that was disassembled showed an impressive amount of mycelium infiltration. A full 77.6% of the chips had been over-run by fungal growth and were in Stage 2. A further 7.8% were in Stage 3, leaving a thin layer of wood chips on the surface of the bed. As can be seen from the image, the deep reddish Stage 2 chips are quite striking next to the dull brownish chips outside the bed. The central, light colored mound under the crossing tape is the area of Stage 3.

Summation

The results of season 1 tests are promising. While much work remains we have demonstrated

an impressive decomposition in a short period of time with very little input of materials or labor. The “trained” organisms do indeed perform well in the wild on the substrates they were intended to consume. While it is assumed the test beds will grow beyond their present confines, no indication has been seen that they have left the inoculated wood chip area or invaded any materials other than wood chips in the surrounding area (reversion to original type).

Based upon what we have seen this season, we will continue to gather data and expand this test to different sites, species, and inoculation techniques. These are promising results worthy of more study. Our hope is to create a solid protocol for mycelial treatment of woodchips, in situ, that is easily replicable and cost- effective. We look forward to sharing with others interested in this pursuit and anticipate new and equally impressive successes in the field of myco-remediation.

Figures:

fig 1 Pleurotus pulmonarius fruiting on woodchips during test:

fig 2: Pleurotus pulmonarius fruiting under jute matting during test:

fig 3, Raw woodchips on top of Stage 2 chips:

fig 4, Stage 3 (digested) woodchips:

Citations

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Appendix A:

Agar formula:

Yield: Approximately 20- 100mm Petri dishes

600 ml H2O
12.5 gr Agar agar
25 gm powdered woodchips from site
1gr Gypsum (buffer)