The fungal degradation of the woody by-products of forest management activities.

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Abstract:

Native, wood-rotting mushrooms were used to accelerate the decay of forest by-products on a remote logging site. The mushrooms were locally collected and conditioned in vitro to recognize wood chips as nourishment. The mushrooms were inoculated into wood chip beds and monitored for five seasons. The mushrooms consumed the wild material, and by the end of the investigation, had converted ~84% of the wood chips into a compost-like material. The control plots lost ~30% of their mass during the same period with no conversion to compost and little loss of structure or resilience. A mild increase in nutrients was detectable in the post-fungal decay product, as was a higher C:N ratio than encountered in natural forest compost (duff). The plausibility of using native wood-rotting mushrooms to decompose logging waste is demonstrated, with reliable starting points for further investigation.

1 Introduction:

Forest management produces large amounts of woody waste material, such as treetops, limbs (slash), and non-merchantable boles (trunks <4" diameter). These cellulosic by-products have little economic value and are often treated as waste material. (Wright, 2012) Standard treatments include: piling on-site for decomposition, burning and hauling off-site for disposal in landfills, or sale as firewood. Slash left on site may take decades or longer to decay (Wagener, 1972). Piles that are not burned intentionally may become 'jackpots' of fuel in a forest fire, increase environmental damage, and risk human life (Battaglia, 2018).

Better management practices are always sought to deal with the demands for increased environmental restoration and reduced wildfire risk. Methods that might reduce carbon emissions and treat waste materials on-site would be desirable to many forest managers. The authors chose to investigate the use of wood-rotting mushrooms to address these wishes and concerns. Ligninolytic saprophytes are ubiquitous fungi that occur in all forest systems and are primary decayers of dead and downed wood in nature. Many in the recent past have suggested their use in remediation, but these techniques have not been well studied (Stamets, 2005). In 2014, we initiated an investigation into the potentials of using native wood-rotting fungi to decay these forest by-products rapidly in-situ.

It is important to note that this study was subject to many of the same constraints as the remedy would if it were widely accepted and implemented, namely funding limitations, limited volunteers to carry out measurements, and the cost of commercial laboratory tests. Thus, our conclusions are preliminary and intended to establish a baseline for future study. We hope that this study sparks interest in the field that will spread like wildfire.

2 Methods:

2.1 Culturing: Native wood-rotting mushrooms were collected in central Colorado and cultured on P.D.A. media. Cultures were generationally conditioned by transfer/amplification onto mediums that contained progressively greater concentrations of the wood chip material into which they would be introduced. Ultimately, the strains were produced onto 3.6 k (8 lb.) blocks of the native wood chips. Two species were chosen for their

vitality and purity in culture: *Pleurotus pulmonarius* (Common Oyster Mushroom), a white rotter mushroom, and *Morchella angusticeps* (Black Morel), a detritivore/mycorrhizal mushroom.

2.2 Experimental design: Two plots with five 1 X 1-meter beds each were constructed at Berrian Mountain Park, a Denver Mountain Park outside of Evergreen, Colorado (Figure 1). The site was chosen because of the excessive fields of woodchips left on-site by a forest fuels treatment the previous year. The plots were excavated, and the bases were leveled. Wood chips were placed and packed to a uniform 12" depth. The layout was four beds in a "four-square" arrangement with a fifth appended to the center of one side to act as a control.

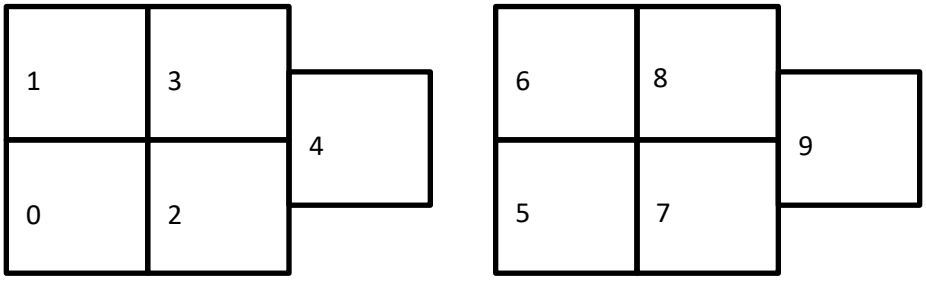

Figure 1. Diagram of the experimental layout with numbered beds 0-4 in plot 1, and 5-9 in the duplicate plot (plot 2)

Four of the beds received one block of spawn with one species for each bed. Plot 1 received Pleurotus p., and plot 2 was inoculated with Morchella a. This seeding yielded an inoculation rate of 37.5 to1 by volume. The beds were treated as follows:
Beds 0 (plot1) and 5 (plot 2): spawn only;

Beds 1 and 6: spawn only, with jute matting from Granite Seed and erosion control in Denver, CO, for moisture retention;

Beds 2 and 7: spawn with nutrients (500 ml fish emulsion & 200 ml humic acid mixed into 1 lt H_2O);

Beds 3 and 8: spawn with nutrients and jute matting;

Beds 4 and 9: control.

2.3 Implementation: The plots were monitored monthly during the growing season for five years, a total of 25 visits over 61 months. The growing season in Evergreen starts in May/June and ends in October/November on average. A random number chart was used to choose the individual beds to be measured on each visit, as is standard for forestry monitoring to vary the disturbance. While this sampling methodology is challenging from a statistical standpoint, this study's overall goal was to investigate whether fungi could be used to mitigate sources of wildland fire ground fuels. Therefore, if we were to sample every bed every month, we would impact the very activity we aim to document. The control beds, lacking any fungal inocula or treatment, were measured on each visit. The beds were monitored for: bed depth, bed temperature, chip moisture content at the moisture horizon, presence of fruit (and species), presence or absence of mycelium, and signs of disturbance. Bed depth was measured with a probe in four random locations on each visit, and the depths were averaged. This method of depth measurement was undertaken to account for the effects of weathering and herbivory. Whether permeated by mycelium or not, chip piles present a prominent moisture horizon, the depth of which was measured on each visit. Samples were taken at this level, and moisture content was determined using National Wildfire Coordinating Group dead-fuel moisture sampling protocols (NWCG, 2019).

The wood chip's structural integrity (Friability) was quantified with a simple screen test. A 1-liter volumetric flask of chips was pressed and agitated against a ¼" screen for 2 minutes, and the weight of the material that passed was compared to the retained fraction. Sample chip friability was tested from a sample bed on day one and both active and control beds on the final day to characterize the treatments' overall effectiveness at chip breakdown.

Wood chip decomposition occurs in definable stages. We chose not to follow the forestry standard of 5 stages of decomposition (Woodall, 2008), because the defining characteristics such as branch attachment and bole deformation were not applicable. Due to the novel character of decomposing wood chips via fungi, we have adopted our own 3 stage system. Stage 1: Raw wood chips; Stage 2: Wood chips involved with the mycelial mat; and Stage 3: Completely decomposed wood chips, indistinguishable from compost or duff. Stage 1 chips persist on the surface primarily due to the desiccating effects of the atmosphere. Once each season, one testbed would be chosen randomly for dissection and reconstruction to measure the decay horizons' characteristics. At the end of the experiment, one control bed was similarly dissected.

3 Results:

The *Morchella* mycelium failed to prosper and was found dead upon excavation by the end of season one. Since Morchella spp. 's life cycle is more complicated than a simple wood rotter, this was a known possibility. The control in Plot 1, bed 4, was rapidly overrun by the *Pleurotus* inoculum. Plot 2 then became the sole control after month 18. The same random testing protocol was then applied to these beds at each monitoring visit.

3.1 Bed Depth. The reported bed depth results from multiple probe measurements (n=4) per visit/per plot in a bed randomly chosen as described above. The below values are season-end measurements with the exception of measurement zero, which was taken on day one. Throughout five growing seasons, the inoculated beds decreased in bed depth from 12" to 4.4" (84.4% decrease) while the control decreased from 12" to 8.5" (30% decrease)

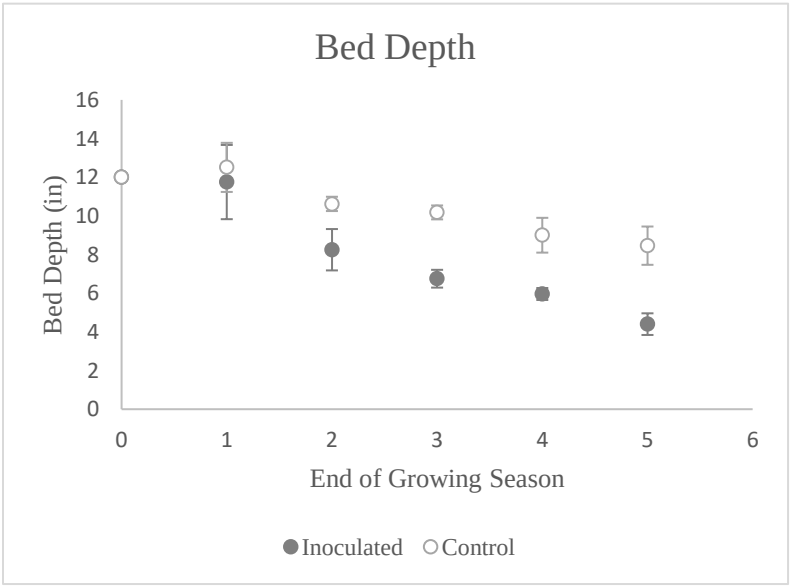

Figure 2. Bed depth of a random bed with spawn vs. the control bed at the end of each growing season.. Error bars represent the standard deviation of n=4.

3.2 Chip Moisture Content at moisture horizon. Data points are taken from the ending of season 2 to ensure that the comparisons are between well-infected and uninoculated wood chips. Season one decay was minor and would reflect a wood chip to wood chip measurement. Figure 3 shows that the inocula had a mild stabilizing effect since the depth to the moisture horizon did not vary as much as the control bed. The moisture content was similar between the control and the inoculated beds, with the inoculated beds retaining slightly more moisture, as illustrated in figure 4. More research would be warranted to determine whether the mycelium acts as a moisture buffer or moisture sponge.

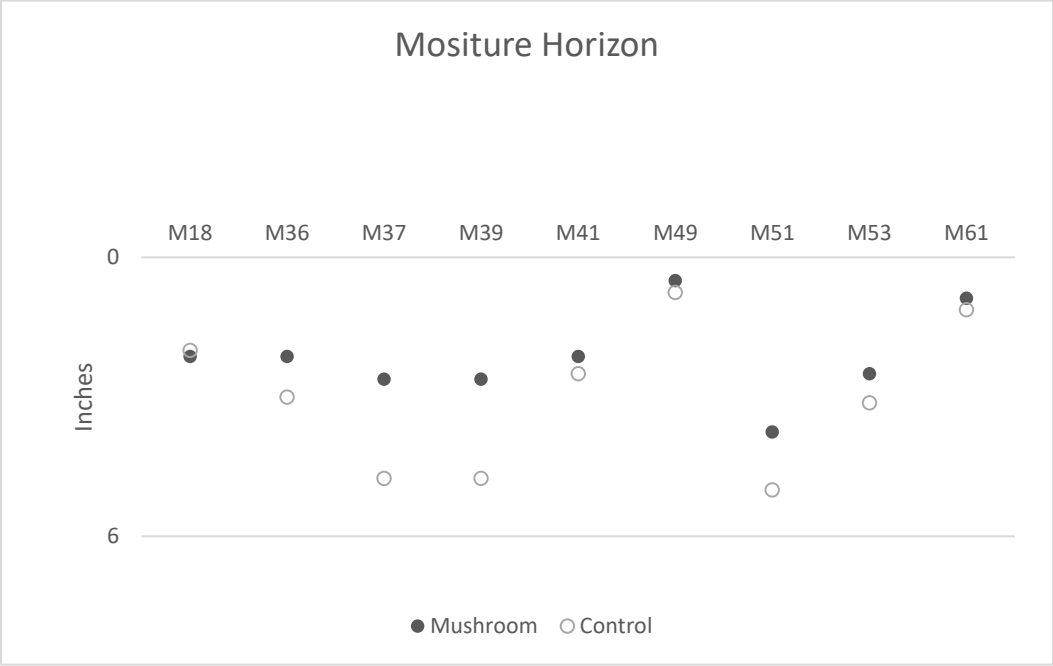

Figure 3. Depth to moisture horizon in inoculated and control treatments over the course of the experiment expressed in months. Data reflects single measurements.

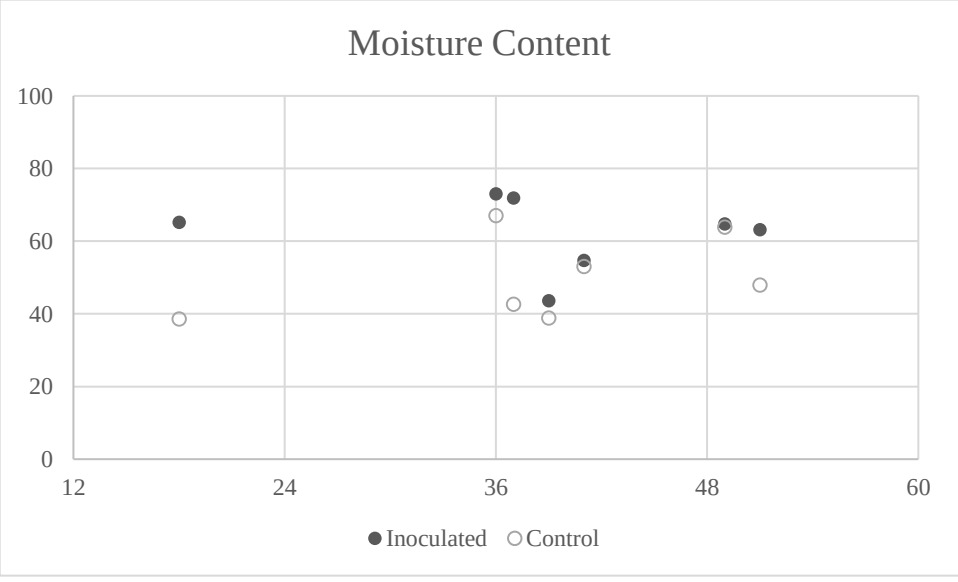

Figure 4. Moisture content over the course of the experiment in inoculated and control treatments expressed in months. Data reflects single measurements.

3.3 Chip Composition. The inoculated beds experienced significant decay throughout the experiment, with the final bed composition 25% stage 1, 15% stage 2, and near 60% stage 3. In contrast, the control was still a majority stage 2, with 0% at stage 3. It is essential to view the composition graphs with the Bed depth results in mind. Season 5 mushroom bed volume is a mere 50% of the season 5 control.

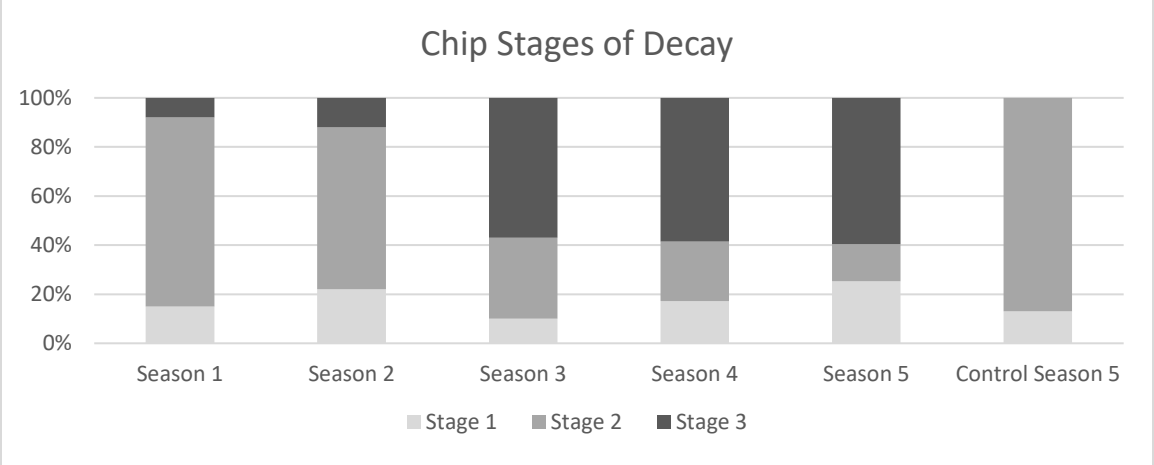

Figure 5 Stages of decay in select beds at seasons end (n=1)

3.4 Friability. (n=2) The measure of chip instability/resilience when subjected to the screen test. The initial run was day 0 raw wood chips from the site. The final run was stage 3 chips from a random mushroom bed and a random control.

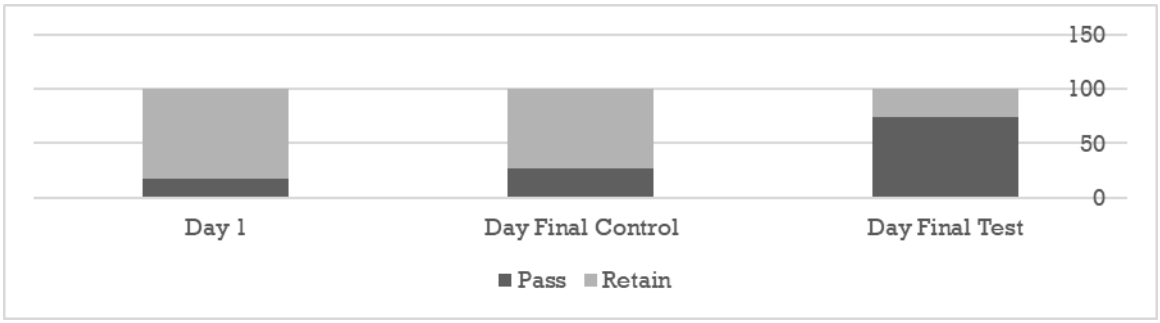

Figure 6 Results of screen tests on wood chips, Day 0 and Day final (n=2)

3.5 Post-decay "compost" composition. Samples of the finished product were sent to soil analytical labs for analysis (n=2) to ensure the material was not harmful to the environment and quantify its nutrient composition.

Metric	Raw wood chips (n=1)	Berrian Compost (n=2)	Conifer O.A. *
C:N	169:1	39.5:1 (s=7.77)	35.5
Ph	4.94	6.8 (s=0.289)	5.7
N	0.279%	0.247% (s=0.024)	0.24%
P	0.010%	0.0335% (s=0.0091)	0.005%
K	0.021%	0.055% (s=0.0077)	0.026%
Org. Matter	89.2%	13.5% (s=4.666)	8.8%

* From: (Buck & St Clair, 2012)
Figure 7 Chemical composition of compost samples

3.6 Fruiting. Fruiting of the inoculated species was confined to the first two seasons. In total, 2.67 k (wet measure) of fruit was harvested. Losses to herbivory are unknown but are certain to have occurred. Each inoculated bed fruited either 3 or 4 times. There was no readily apparent difference in collected yield from any bed's nutrient treatment or matting. No conclusion can be drawn as to the efficacy of this treatment as a mushroom producing activity.

3.7 Topsoil Production. An unintended by-product of this test was discovering an organic/mineral intermix layer beneath the inoculated beds. The "topsoil" was present under each testbed and is a somewhat expected result of organic decay above a D.G. (Decayed Granite) soil type. The depths under the inoculated beds averaged 1.562" (n=4, sd= 0.38) and 0.18" (n=4, sd= 0.062) beneath the controls. No intermix layers were present outside of the two testbeds (0.0", n=2).

Figure 7 Topsoil Plot 1, Bed 1, 5/1/2020

4 Discussion:

Our candidate mushroom species successfully decayed the majority of their woodchips within the time frame of this study. The inoculated plots' total degradation calculates to 84.4% (36% conversion of the final 40% bed depth). The control yielded a 30% mass decrease (0% production of Stage 3 and 30% bed depth loss), perhaps due mostly to herbivory, compaction, and pre-decay. This initial study's sampling methods were insufficient to determine if nutrient supplementation affected the mushroom's decay rate. Although fruiting does not appear to be associated with this decay rate, it is compelling evidence that the mushrooms will successfully overwinter in wood chip piles.

Both treated and control beds increased in depth throughout the first season, but there is no statistical significance to this, and it is probably a by-product of the disturbance of inoculation and mechanical side-effects of moisture penetration on a pre-compacted pile of wood chips. The conversion of woodchips into decayed "compost" by the *Pleurotus* appears substantially complete by Season 4. The control beds failed to reach Stage 3 decay within the five years of the trial and lost 30% of their original volume. The rapid decay of the inoculated beds might, in part, be attributed to the greater moisture capacity of chips involved with mycelium, as opposed to raw wood waste. The increased moisture capacity is likely achieved both by the de-lamination of the cellulosic structure, affected by the digestion of the compound lignin, and the fungal organisms' internal

cytoplasm. The only time the moisture horizons of test chips and the control chips approached equity was around Month 49, following a winter with nearly 12" of moisture (70% of average annual total rainfall. Month 49 was also the month where rainfalls at this location returned to average after three years of above-average moisture (Season 1, + 8.8"; Season 2, +2.7"; Season 3, +7" (CocoRaHS, 2020))

The friability test demonstrates the state of decay that was attained during this study period. Wood is an extremely recalcitrant compound, and this test demonstrates the completeness with which the mushrooms deconstruct the cellulose/lignin/hemicellulose complex.

Our chemical analysis of the decay by-products demonstrates the similarity to natural forest floor duff, formed within an accelerated period. It also demonstrates a modest concentrating of the nutrients' potassium and phosphorus, with little change in the overall percentage of nitrogen. Of interest is the higher concentration of carbon in the end product as compared to natural forest litter. Further investigation of white rotters' compost carbon content (lignin consumers) versus the brown rotters (cellulose consumers) would be desirable if looking into this technique as it relates to carbon sequestration. Lignin can compose 15% to 40% of forest "soils" (Krishna & Mohan, 2017), holding soil carbon in a stable form. Therefore, brown rotters would be expected to exhibit an even greater carbon density in their final product than the result of the white-rot from our *Pleurotus* mushrooms.

The encountered topsoil production would be expected to be transitory as the surface organics return to the carbon cycle either as the flesh of organisms or the off-gassing of carbon oxides. It is included as a potential for further study to determine if this dwell time will be measured in years, decades, or longer. An understanding of the impacts of this novel soil formation on the forest floor community would also seem desirable from an ecological standpoint.

6 Conclusion:

This technique is a simple, ecologically balanced prescription for slash disposal in montane/ sub-alpine forests. Further work needs to be done to streamline these techniques and application rates, but this study establishes a reasonable baseline for future inquiry. The ease of propagation and application of native fungal organisms opens a new door to many different applications in forest management and conservation. The authors hope that this work will help others as they investigate the use of native ecology to heal ecosystem disturbances economically, rapidly, and safely.

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