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

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1 Introduction:

Forest management activities produce large amounts of woody waste material, such as treetops, limbs (slash), and non-merchantable boles (trunks <4" diameter.) Estimates are that over 60 million metric tonnes of waste wood are produced annually as of 2020, and that amount is expected to rise. (Zimmer 2018) These cellulosic by-products have little economic value and are often treated as waste material. (Wright 2012) Up to 30 million dry tonnes of this waste may be left in insitu annually. Currently, practitioners are increasingly looking towards biomass as a fuel, or biofuel conversion to deal with this excess waste. (Pokharel 2019) Other common treatments include: piling on site for decomposition, burning and hauling off-site for disposal in landfills, or sale as firewood. Piles that are not burned intentionally may become 'jackpots' of fuel in a forest fire, increase environmental damage, and risk human life. (Battaglia 2018) Slash left on site may take decades or longer to decay (Wagener 1972).

However, those concerned with staving off the effects of climate change would prefer no additional carbon be released into the atmosphere, regardless of the source. (PFPI 2011) Better management practices are, and should be, continually sought to deal with the demands for increased environmental preservation and reduced wildfire risk. Methods that might reduce carbon emissions and treat waste materials on-site would be desirable to many forest managers. The reduced cost of not having to haul waste from remote sites deserves the attention of all in the field who are concerned with the staggering costs of forest mitigation on a global scale (Austin 2020).

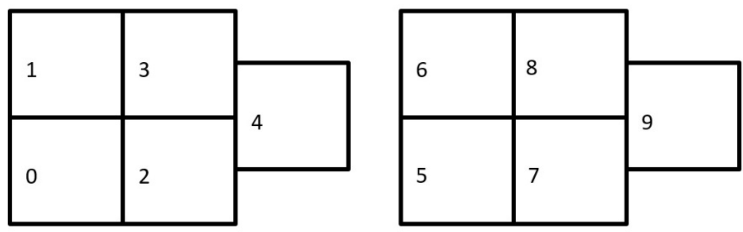
24 The authors chose to investigate the use of wood-rotting mushrooms to address these wishes and concerns.
25 Ligninolytic saprophytes are ubiquitous fungi that occur in all forest systems and are primary decomposers of
26 dead and downed wood in nature. Many populist reports in the recent past have suggested their use in
27 remediation, (Stamets 2005) but these techniques have not been well studied. The use of basidiomycetes in
28 bioremediation had been investigated since the early 1990s, but not on pure wood waste materials (Kirk 1995).
29 Literature review could find only one pilot study on the use of wood rotters to reduce waste wood (Croan 2000).
30 Our 5 year effort predated it in initiation by 4 years. (O'Donnell 2019). So, we were unaware of it when we
31 started. In 2014, we began our investigation into the potentials of using native wood-rotting fungi to decay these
32 forest by-products in situ, potentially rapidly.

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

38 2 Methods:

39 **2.1 Strain selection:** Native wood-rotting mushrooms were collected in central Colorado. Our initial candidate
40 species were: Pine Oyster (Pleurotus *pulmonarius*), King Agaricus (*Agaricus silvicola*), Box Elder Oyster
41 (*Hypsizygus tessulatus*), Common puffball *(Lycoperdon perlatum*), King Stropharia (Stropharia ruggoso-
42 annulata), and the Black Morel (*Morchella angusticeps)* They were cloned by dissection and cultured on P.D.A.
43 media: 250 gr potatoes blanched in 1000 ml H_2O, strained and returned to 1L, and finished with 20 g of
44 bacteriological grade agar and 10 g of glucose. The medium was autoclaved for 25 min at 121°C, then decanted
45 into 100 mm petri dishes. Cultures were generationally conditioned by transfer/amplification onto mediums that
46 contained progressively greater concentrations of the wood chip material into which they would be introduced.
47 **2.2 Culturing:** Initially, the strains were selected for vigor on the PDA petri dishes, and transferred to 2 quart
48 mason jars with micropore filters containing 90 g of a bone dry sawdust and wood chip mix collected at the site

49 where final introduction to the test plots would occur, along with 35g organic rye grain, 1g lab-grade calcium
50 sulfate, and 137ml of water for a target of 55% - 60% moisture. Jars were autoclaved at 121°C for 45 minutes
51 and cooled in the autoclave overnight. Jar inoculations were made directly from those cultures selected for
52 vigor. (We rejected the King Stropharia at this stage per the suggestion of Vera Stucky Evenson, curator of
53 fungi at Denver Botanic Gardens, over fears that they were an invasive species in Colorado). Inoculated jars
54 were incubated in subdued light at room temperature for 21 days. The chosen strains were amplified, 1 to 5,
55 once more into jars with the same medium mixture. Finally, the two strains chosen for vigor and mycelial
56 density were inoculated into Phoenix mushroom bags with a medium of 1000 g woodchips and sawdust, 166g
57 of rye, and 1.25L of water for a moisture content of ~60%. Two species were chosen for their vitality and purity
58 in culture: the common oyster mushroom a white rotter basidiomycete mushroom, and the black morel, a
59 detritivorous/mycorrhizal ascomycete mushroom. The morel was chosen because of its extreme vigor under
60 these growth conditions and the similarity of our inoculated plots to pilot commercial production strategies
61 practiced in China (Zhu 2008) and the United States (Kuo 2008), where the requisite saprophytic stage, as well
62 as a mycorrhizal companionship were both possible at our remote inoculation site. (Liu 2017)
63 **2.3 Experimental design:** Two plots with five 1 X 1-meter beds each were constructed at Berrian Mountain
64 Park, a Denver Mountain Parks property outside of Evergreen, Colorado (Figure 1). The site was chosen
65 because of the massive fields of woodchips left on-site by a forest fuels treatment the previous year. The plots
66 were excavated, and the bases were leveled. Wood chips were placed and packed to a uniform 12" depth. The
67 layout was four beds in a "four-square" arrangement with a fifth appended to the center of one side to act as a
68 control.
69 Figure 1



70 Test Site Design

71 Four of the beds received one block of spawn with one species for each plot. Plot 1 received common oyster,
72 and plot 2 was inoculated with black morel This represents a rate of seeding of 37.5 to1 by volume. The beds
73 were treated as follows: Beds 0 (plot1) and 5 (plot 2): spawn only; Beds 1 and 6: spawn only, with jute matting
74 from Granite Seed and erosion control in Denver, CO, for moisture retention. Beds 2 and 7: spawn with
75 nutrients (500 ml fish emulsion & 200 ml humic acid mixed into 1 L H_2O). Beds 3 and 8: spawn with nutrients
76 and jute matting. Beds 4 and 9 were established as controls. Steel fence posts delineated the plots and caution
77 tape was wrapped around it to prevent human interaction. The sites were signed with information on the project.
78 **2.3 Implementation:** The plots were monitored monthly during the growing season for five years, a total of 25
79 visits over 61 months. The growing season in Evergreen, CO, starts in May/June and ends October/November
80 on average. A random number chart was used to choose the individual beds to be measured on each visit, as is
81 standard for forestry monitoring, and to vary the disturbance. The control beds, lacking any fungal inoculum or
82 treatment, were measured on each visit. The beds were monitored for: bed depth, bed temperature, chip
83 moisture content at the moisture horizon, presence of fruit (and species), presence or absence of mycelium, and
84 signs of disturbance. Bed depth was measured with a probe in four random locations on each visit, and the
85 depths were averaged. This method of depth measurement was undertaken to account for the disturbance of
86 weathering and herbivory. Chip piles present a prominent moisture horizon (MHoriz), whether permeated by
87 mycelium or not, the depth of which was measured on each visit. Samples were taken at this level, and moisture
88 content was determined using National Wildfire Coordinating Group dead-fuel moisture sampling protocols
89 (NWCG 2019). All measurements were recorded on paper and a custom app designed to collect this study's
90 data. All monitoring sheets were signed by a monitoring lead and witness (if present).
91 The wood chip's structural integrity (Friability) was quantified with a simple screen test. A 1-liter volumetric
92 flask of chips was pressed and agitated against a ¼" screen for 2 minutes, and the weight of the material that
93 passed was compared to the retained fraction. Sample chip friability was tested from a sample bed on day
94 one(n=2) and both active and control beds (n=4) on the final day to characterize the treatments' overall
95 effectiveness at chip breakdown.
96 Due to the novel character of decomposing wood chips via fungi, we have adopted a 3 stage system suggested

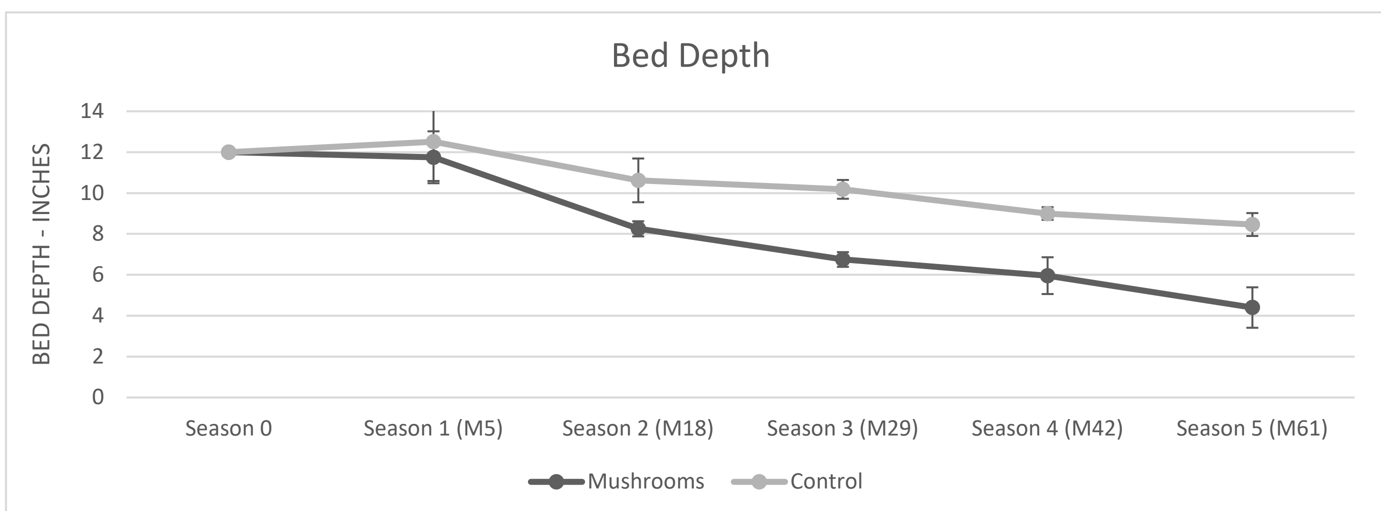
97 by.Bunnell and Houde (Bunnell 2010) Stage 1: Raw wood chips; Stage 2: Early decay (Wood chips involved
98 with the mycelial mat); and Stage 3: Late stage decay (Completely decomposed wood chips: compost or duff.)
99 A layer of Stage 1 chips persist on the surface of all beds primarily due to the desiccating effects of the
100 atmosphere. Once each season, a test-bed would be chosen randomly for dissection and reconstruction to
101 measure the decay horizons. At the end of the experiment, one control bed was similarly dissected as well.

102 ## 3 Results:

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

107 **3.1 Bed Depth.** The reported bed depth results from multiple probe measurements (n=4) per visit/per plot in a
108 bed randomly chosen as described above. The below values are season-end measurements with the exception of
109 measurement zero, which was taken on day one. Throughout five growing seasons, the inoculated beds
110 decreased in bed depth from 12" to 4.4" (84.4% decrease) while the control decreased from 12" to 8.5" (30%
111 decrease). This represents an over 50% comparative increase in mass reduction over the course of the test (p=
112 0.000033)

113 Figure 2



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115 . **3.2 Chip Moisture Content at moisture horizon.** Data points were taken from the ending of season 2 to

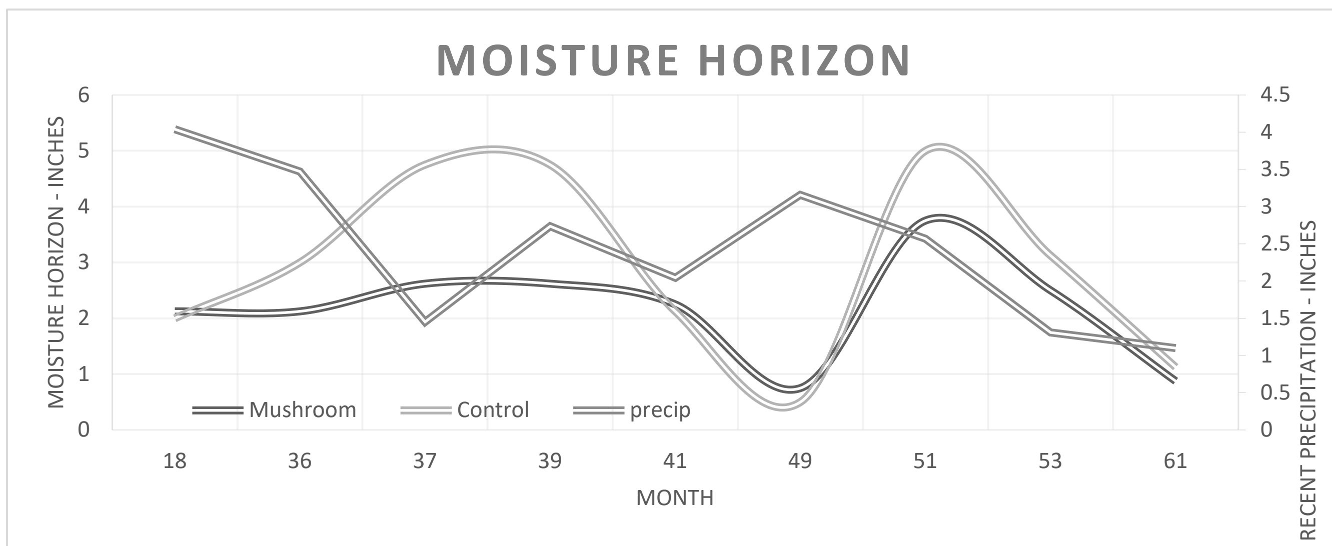
116 ensure that the comparisons are between well-infected and uninoculated wood chips. Season one decay was

117 minor and would most probably reflect a wood chip to wood chip measurement. Figure 3 Plots the depth of the

118 moisture horizon overlaid with the precipitation from the last visit. Figure 4 illustrates the substrate moisture

119 percentage at the moisture horizon (MHoriz) also plotted against the precipitation from prior visit.

120 Figure 3.



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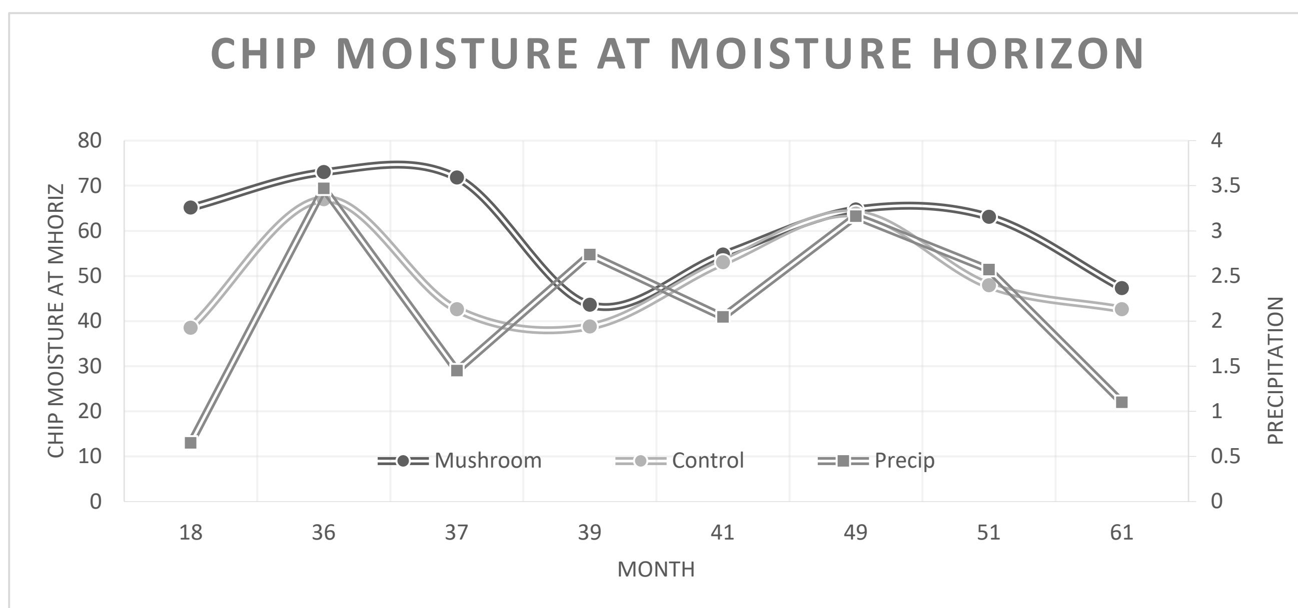
122 Figure 4.



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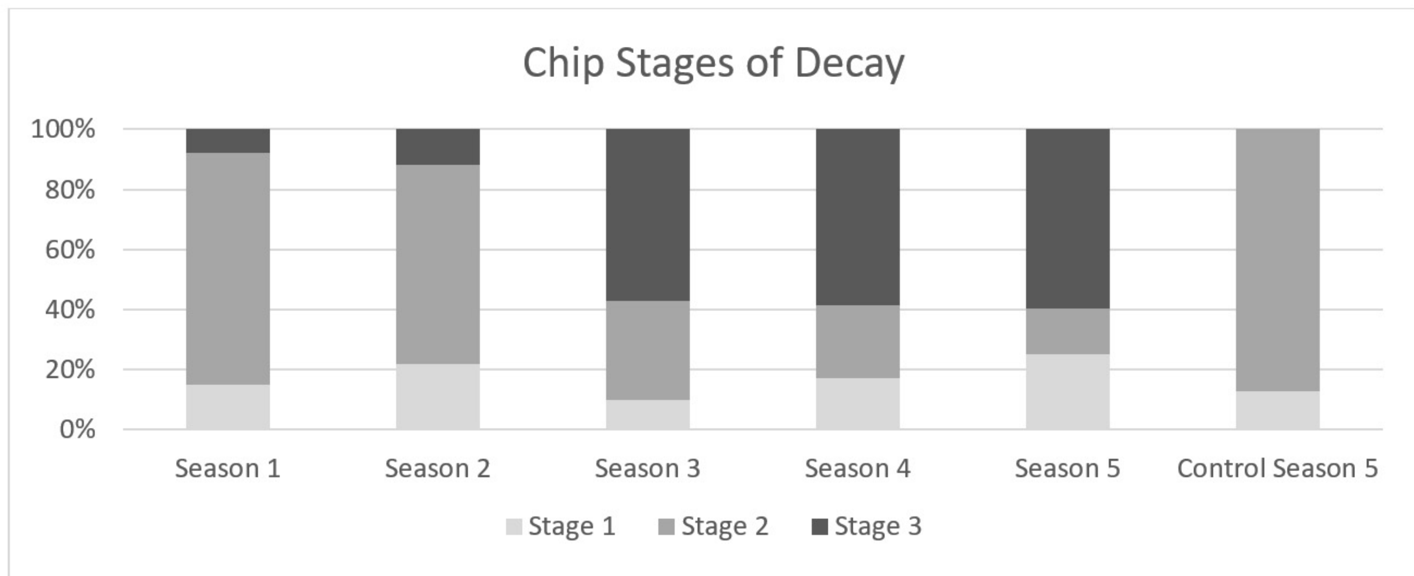
125 **3.3 Chip Composition.** The inoculated beds experienced significant decay throughout the experiment, with the

126 final bed composition 25% stage 1, 15% stage 2, and nearly 60% stage 3. In contrast, the control was still

127 composed of a majority stage 2, with 0% at stage 3. This would be consistent with expected rates of decay in a

128 Colorado montane forest.

129 Figure 5.



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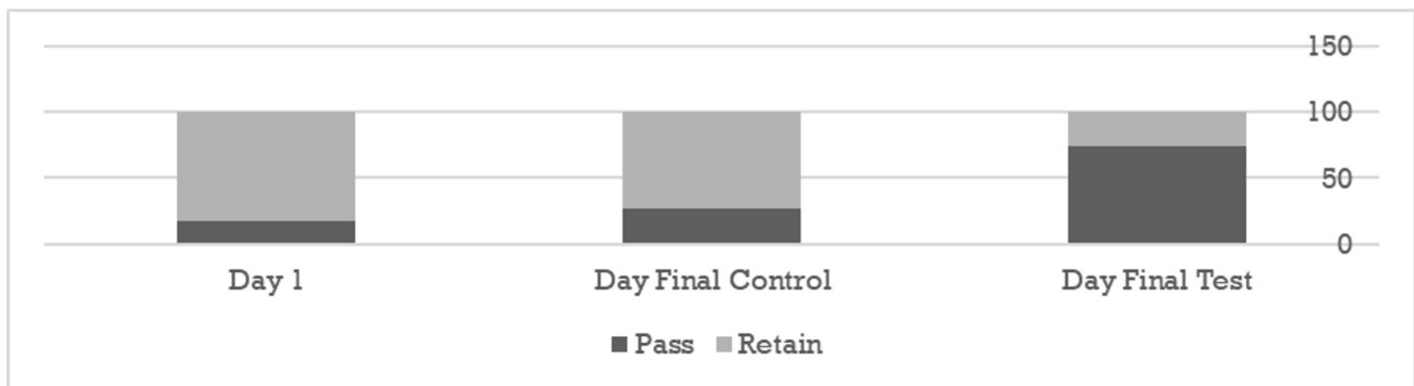
131 **3.4 Friability.** (n=2) The measure of chip instability/resilience when subjected to the screen test. The initial run

132 was performed on day 1 with raw wood chips from the site. The final run was stage 3 chips from a random

133 mushroom decomposed bed and stage 2 chips from a random control.

134 Figure 6.



135 **3.5 Post-decay "compost" composition.** Samples of the finished product were sent to soil analytical labs for
136 analysis (n=2) to ensure the material was not harmful to the environment and to quantify its nutrient
137 composition.

138 Table 1 Chemical composition of compost samples

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%

139 * From Buck and St Clair

140 **3.6 Fruiting.** Fruiting of the inoculated species was confined to the first two seasons. In total, 2.67 k (wet
141 measure) of fruit was harvested. Losses to herbivory are unknown but are certain to have occurred. Each
142 inoculated bed fruited either 3 or 4 times. There was no readily apparent difference in collected yield from any
143 bed's nutrient treatment or from the presence of matting. No conclusion can be drawn as to the efficacy of this
144 treatment as a potential mushroom producing activity.

145 # 4 Discussion:

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

153 Both treated and control beds increased in depth throughout the first season, but there seem to be no particular
154 significance to this, and it is probably a by-product of the disturbance of inoculation and mechanical side-effects

155 of moisture penetration on a pre-compacted pile of wood chips. The conversion of woodchips into decayed
156 "compost" by the Oyster mushroom appears substantially complete by Season 4. The control beds failed to
157 reach Stage 3 decay within the five years of the trial and lost 30% of their original volume. This rapid decay of
158 the inoculated beds might, in part, be attributed to the greater moisture capacity of chips involved with
159 mycelium, as opposed to raw wood waste. The increased moisture capacity is likely achieved both by the de-
160 lamination of the cellulosic structure, its fibrous matrix a by-product of the digestion of the compound lignin,
161 and the fungal organisms' internal store of cytoplasm. This greater moisture capacity will likely also aid the
162 functions of secondary decomposers and bacteria, all contributors to the ultimate decay of the wood chips. The
163 graphs clearly show that in times of moderate precipitation (< ~2.5 in) the difference in moisture holding
164 capacity of inoculated chips is noticeably greater than in the control chips. In times of heavy precipitation, all
165 soils measured similarly, being close to saturation. The times the moisture horizons of test chips and the control
166 chips approached equity was around Month 49, following a winter with nearly 12" of moisture (70% of average
167 annual total rainfall.) and Month 49, a month of high rainfall after which, precipitation returned to average after
168 three years of above-average moisture (Season 1, + 8.8"; Season 2, +2.7"; Season 3, +7" (CocoRaHS 2020)) .
169 The friability test demonstrates the completeness of decay that was attained during this study period. Wood is
170 an extremely recalcitrant compound, and this test demonstrates the extent to which the mushrooms can
171 deconstruct the cellulose/lignin/hemicellulose complex.
172 Our test mushrooms successfully converted over 60% of the bulk mass of our test beds into compost over the
173 course of the study. Chemical analysis of the decay by-products demonstrates the similarity of this compost to
174 natural forest floor duff, albeit formed within an accelerated period. It also demonstrates a modest concentrating
175 of the nutrients' potassium and phosphorus, with little change in the overall percentage of nitrogen. Of interest is
176 the higher concentration of carbon in the end product as compared to natural forest litter. Further investigation
177 of white rotters' compost carbon content (lignin consumers) versus the brown rotters (cellulose consumers) as it
178 selates to carbon sequestration is currently under investigation. Lignin can compose 15% to 40% of forest soils
179 (Krishna and Mohan 2017), holding soil carbon in a stable form. Therefore, brown rotters would be expected to
180 exhibit an even greater carbon density in their final product than the result of the white-rot from our common

Oyster mushrooms.

6 Conclusion:

This technique is a simple, ecologically balanced prescription for waste wood 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 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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