Fungal Degradation of the Woody By-products of Forest Management Activities Second Year Report, 2016 (Y2)

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Project partners:

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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 are left on the forest floor by several types of mitigation treatments including mastication, hydro-axing and whole-tree 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 take decades to degrade naturally, and have a severe impact on the forest understory. Estimates of the time woody debris takes to decay naturally vary wildly, as do the conditions of that decay. Full decay of Coniferous tree stems in a natural forest setting in Colorado may take from 57 to 124 years (Russell etal., 2014).

This investigation is being conducted to study the possibilities that the techniques of myco-remediation offer promise as a tool for forest management practitioners, by allowing for the post-harvest treatment of woody byproducts. We initially sought to test the efficacy of using native mushroom species to digest wood chips and document the procedures and their results. This investigation seeks to create a 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 $(C_6H_{10}O_5)_n$, and lignin $(C_9H_{10}O_2, C_{10}H_{12}O_3, C_{11}H_{14}O_4)_n$, 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, offer the potential to degrade

large quantities of material. These treatments can be used to accelerate the natural decay and breakdown of woodchips left from tree mitigation treatments and accelerate the return of understory communities.

- 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 (Lignicolous Fungi) as the starting point in our efforts to generate mushrooms adapted to wood chips from our test sites. 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. This leads to a completely different form of “composting” that can occur in a pure wood chip environment without the need of further human activity.

Year two (Y2) consists of continued monitoring of the original test beds, and the recruitment of new species for further inoculations in Year 3 (Y3). Collaboration with Denver Botanic Gardens and the Colorado Mycological society gave us the opportunity to collect live tissue samples from several species for cultivation at our facility. We are currently working with 2 strains of Pleurotus pulmanarius; one strain of Pleurotus populinus; Gleophyllum sepiarium, the gilled polypore; Connopus acervatus (formerly: Gymnopus); and Neolentinus ponderosus, the Trainwrecker.

2. Test Site

The test is taking place on Denver Mountain Parks (DMP) property in Jefferson County, Colorado. Two species were chosen by the DMP Forester and Naturalist for this test. 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	P. pulmanarius w/ nutrients & Jute	M. angusticeps w/nutrients & Jute	Bed

3	matting	matting	8
Bed 4	CONTROL	CONTROL	Bed 9

Jute matting was used to assess 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".

4. Monitoring

The beds were checked 4 times over 5 months. The dates are counted in months from initial inoculation for ease of analysis (Month 13 to Month 18). At each visit a visual check was made for fruiting bodies, and samples were taken and weighed when present. Bed depth and temperature was measured, as were the ambient weather conditions and soil temperature. To minimize intrusion, a random number chart was used to select 2 of the 5 beds at each site to measure each visit. On month 16 it was discovered that the control plot (#4) of the Pleurotus beds had become completely infested by our mycelium, ending its usefulness as a control. A lack of fruit and, on examination, mycelia; led to the use of the Morchella beds as substitute controls, and are used in the season's final calculations .
Monitoring Dates are as follows: 6/14/2016 (Month 13), 7/08/2016 (Month14), 9/09/2016 (Month 16), 11/14/2016 (Month 18)

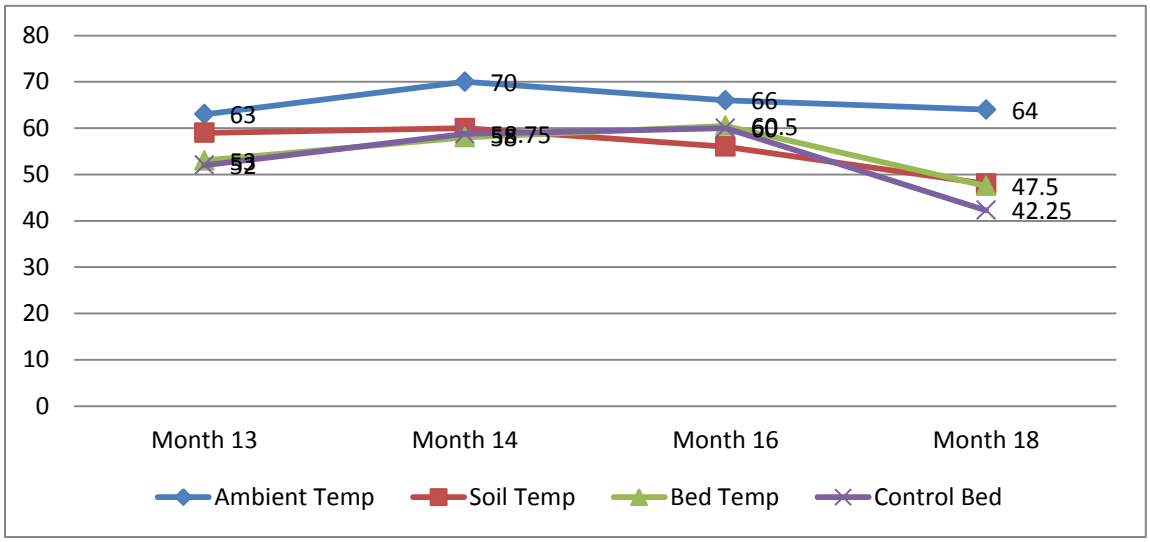
The final monitoring was conducted on November, 14th 2016 (Month 18). At this time final measurements were made and samples taken for an end of season sample collection. The random chart was also used to select one bed for disassembly in order to measure the extent of mycelial spread. The bed was excavated with trowels, one inch at a time beneath a grid to ultimately measure the depth and extent of infiltration.

5. Results

This season again had fruiting present on each test bed on the P. pulmonarius site. Each bed fruited at least once with one bed fruiting twice. Y2 was a dryer year than Y1, but the data shows that digestion of the woody material continues more or less independently from the occurrence of fruit (mushrooms).

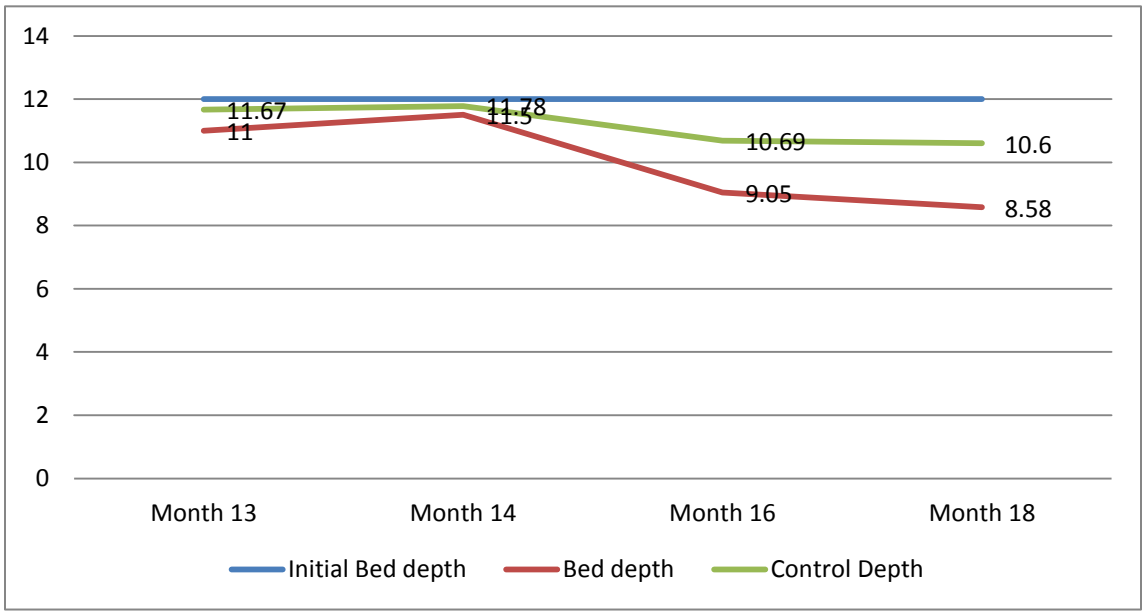
a)Raw Data:

Table 1: Monitoring Temperature Data



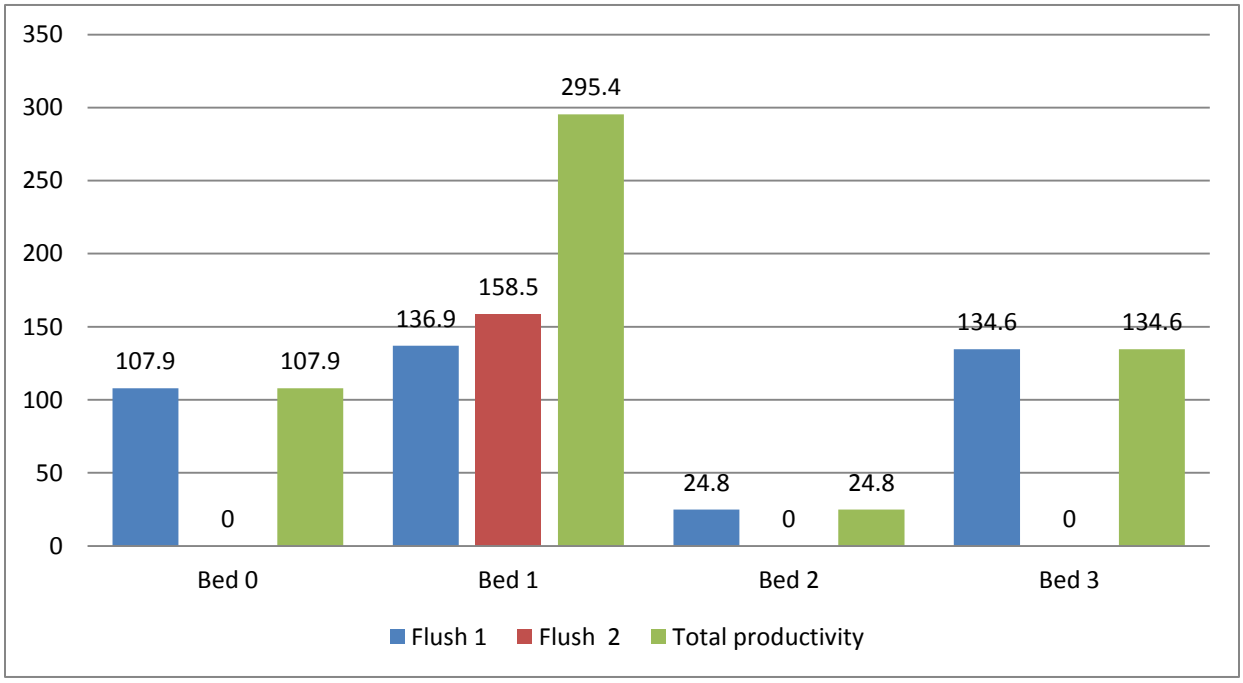
Soil temperature remains fairly constant,(59°F- 60°F June-August), becoming cooler after September (56°F - 48°F) when the region had begun a cool down. Bed temperatures are fairly constant and there was little difference between the test beds and controls. Any increase in bed temperature would be expected to indicate the presence of bacterial decomposition. Enzymatic effects of the fungal decay should be keeping bacterial contamination to a minimum. The only conspecifics noted were slime mold scleroteum (sp. unknown) documented each year at disassembly in the Fall. (See: fig 5)

Table 2: Bed Depth Data



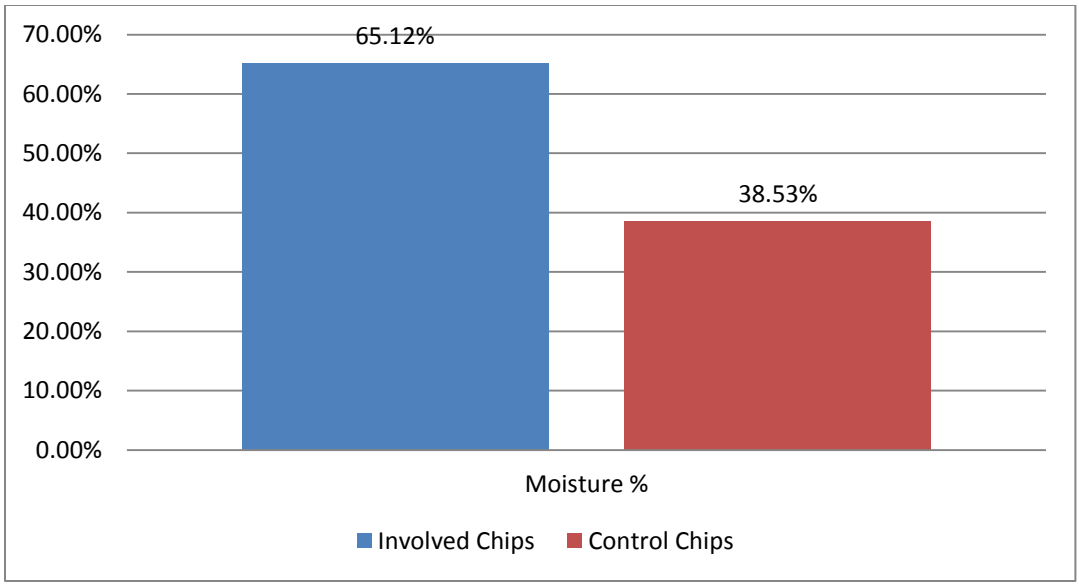
Year 2 (Y2) begins to show a definite digestion of the woodchip beds. Average bed depth reached 71.5% of the starting value, while the control plots measure 88% of the starting value. This is a good demonstration that the desired degradation continues despite the lack of fruiting. The switch from the attached control (Plot #4) to the Morchella beds (which began with the same conditions) occurs at the Sept (Month 15) monitoring visit. A 2014 experiment (Boberg 2014) on saprobic fungi in leaf litter demonstrated the propensity of these fungi to only partially digest a food source and then migrate to a fresh supply taking Carbon and Nitrogen with it. This appears to be what is happening in the contiguous control plot, which at that date was discovered to be fully over-run with the Pleurotus mycelium, from the adjacent beds.

Table 3: Fruiting Data (in grams, wet weight)



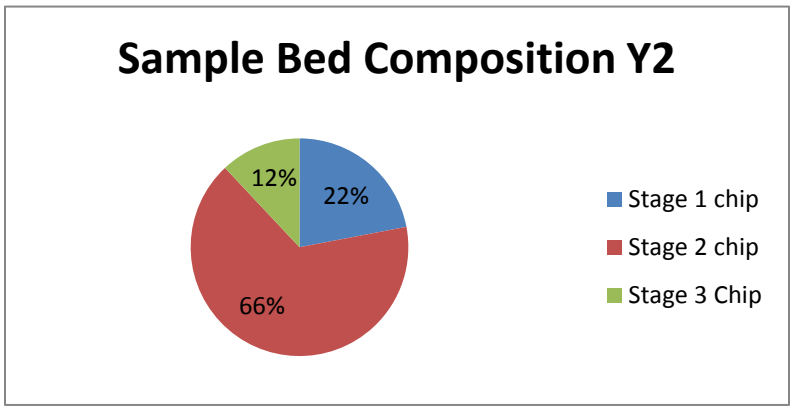
Fruiting was about 10% of the first year at 267.3 grams (wet weight) total. Fruiting is stimulated by moist conditions on the surface of the medium, followed by a period of warmth, a set of conditions that was just not met often in Y2. The moisture horizon in the woodchips this season sat around 2.8" below the surface of the woodchips in both active and control plots. (Active: 2.85" avg., Control 2.75" avg.).

Table 4: Moisture Content of chips below Moisture Horizon



Woodchips that are fully over-run with mycelia hold 59% more moisture as the controls. This is a well-known property of fungi (Guhr, etal 2015). This moisture retention property of the fungal growth process is vital to nutrient availability and transport, and is seen as key to the myriad potential benefits of these treatments. It is possible and likely that this living mass will be more resistant to ignition, an important property for any material left alone in the forest. This will be a major subject for further investigation.

Table 5: Chip Degradation



At the end of each season, one sample bed is dissected, layer by layer to measure the areas and completion percentages of the degradation. This was done at the end of the first season and appeared to have little effect on the continuation of the degradation processes. In Y2 that bed was Bed #1. This chart shows, roughly, the composition of the chips in terms of degradation stage. 78% of the woodchips are now actively involved in degradation.

In the first year we postulated 3 chips stages. Stage 1: Raw woodchips. Stage 2: Overrun by

mycelia but still recognizable as a woodchip. Stage 3: complete degradation and reduced to a "compost" like structure. We noticed that Stage 2 chips were actually harder, and more resistant to screen abrasion than the Stage 1 Raw chips. We have now seen there are two phases to Stage 2. Stage 2, phase 1 is an involved, orangey chip that is similar in size and shape to a Raw chip, but more resistant to abrasion. Stage 2, phase 2, are identical in appearance to phase 1, but have lost strength and are spongy to the touch. (See fig. 2) Stage 2 , phase 2 are extremely friable; they retain shape and appearance of a woodchip but crumble in your hand. More lab tests will be needed to get a precise qualification of these stages.

b) Nutrient qualities of wood chips and the final products of decay

Y2 allowed us to produce enough material for nutrient testing of the finished product, and also saw a modest increase in funding that made these tests possible. Raw woodchips and finished "compost" samples were delivered to Colorado Analytical Laboratories for chemical analysis. We have been encouraged to look not only at the benefits of retaining nutrients within the landscape, but also to begin investigation of Carbon sequestration within the decay (D.J. Lodge personal communication, August 17, 2016). Cellulose is approximately 40% Carbon by weight ($C_6H_{10}O_5$). Great interest surrounds the potential of keeping Carbon locked into organic compounds and out of the atmosphere. Currently, we are investigating the creation of carbon sequestration credits that can be sold in carbon markets to help offset the cost of forest mitigation treatments. This suggests a further avenue for quantification of forest services as a valuable economic resource.

Organic Nitrogen is an essential nutrient for plants. Rocky Mountain forests are noticeably poor in organic Nitrogen and as such it is bound tightly by the organisms that use it and acts as a limit on growth. Fire, which is a natural component of these forests, destroys roughly 100% of available organic Nitrogen in its fuel by converting it directly into N_2 gas during wildfire events. Nitrogen comprises over 70% of our atmosphere, but is unavailable for biological processes (inorganic). While mushrooms do not "fix" Nitrogen (as do some bacteria), they are capable of collecting and "hording" it. Once the mushroom cycle is completed within a medium, this Nitrogen becomes available as a nutrient for succession (plants).

The Carbon to Nitrogen Ratio is a measure of the proportions of these two elements in a material, in this case: compost. The C/N ratio will determine whether the compost will act as a Nitrogen source (providing nutrients for plant growth) , or a Nitrogen sink (actually pulling available Nitrogen from the environment around it). The standard ratios needed for a Nitrogen source are 15:1 to 20:1 (15-20 times as much Carbon as Nitrogen).

Raw woodchips in this experiment had a C/N ratio of 169:1. The compost at the end of digestion had a C/N ratio of 34:1. This is essentially the same as the natural C/N ratio of organic detritus (Duff) in a Rocky Mountain mixed conifer forest (Buck,2012). The Nitrogen in the "finished" sample was predominantly Ammonia (ammonia-N/nitrate-N ratio; 10.7:1), as would be expected as the by-product of the fungal metabolism. In this form it is available to nitrosomonas and nitrobacter bacterias to fuel a normal Organic Nitrogen cycle.

Ph is the measure of acidity or alkalinity within a medium. It also affects the availability of nutrients. Raw woodchips, with a Ph of 4.94 is acidic and may affect the uptake of Phosphorus by plants. The finished "compost", with a Ph of 7.01 is neutral and perfectly desirable in terms soil chemistry. The Ph of forest duff in natural mixed conifer forests is around: 5.7 (Buck, 2012), less acidic than the raw woodchips but more acidic than the finished mushroom compost.

The tests demonstrate that the mushroom degraded material is a genuinely valuable nutrient supply and is succeeding in terms of maintaining precious forest nutrients that would either be consumed by fire, or hauled away by loggers in standard forest mitigation treatments.

Summation

The results of Y 2 tests are impressive. The mushrooms have demonstrated the ability to "over-winter" unaided in their beds. They have demonstrated good resistance to disturbance (Deer and Squirrel digging, Deconstruction for sampling and re-building, etc.) and have pioneered at a measured pace into new areas (overtaking of control plot). They have shown greater decomposition than the control chip piles, and promise a route to transform what was previously considered a waste material into a usable nutrient compost; without the heat and subsequent danger of spontaneous combustion found with bacterial composting techniques. Again, we have noticed no significant difference between covered and uncovered, or nutrient enriched or plain woodchips. This is helpful allowing for treatment with minimal effort.

We are continually impressed by the vigor, adaptability and ubiquity of these humble organisms and look forward to the next round of tests and the continued monitoring of our first square meters of "myco-forestry" in action.

Acknowledgements:

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Further, Thanks to: Vera Everson PhD., Beth Nielsen, Emma Ravage, D. Jean Lodge PhD., James Weiser, Joseph Hansen and Carol Ekarius for their labor and guidance without whom we'd still be wondering: "is this even possible?"

Figures:

fig 1 Pleurotus pulmonarius fruiting on woodchips during test:

fig 3, Stage 2 phase2 on Stage 2 phase 1 chips:

fig 4, Stage 3 (digested) woodchips:

fig. 5 Slime Mold scleroteum from season two final monitoring

Citations

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Appendix A: Lab results

Colorado Analytical LABORATORIES, INC.

LABORATORY ANALYSIS REPORT

REPORT TO: JEFF RAVAGE

BILL TO: COALITION FOR THE UPPER SOUTH PLATTE
P.O. BOX 726
LAKE GEORGE CO 80827

LAB NO: 27296.02
DATE RCVD: 11/14/16
REPORTED: 12/1/16
PO NO.:

PROJECT: FUNGAL DEGRADATIONI STUDY

SAMPLE ID: **RAW CHIP SAMPLE**
MATRIX: COMPOST
SAMPLE DATE: 11/14/16

	AS RECEIVED BASIS	DRY MATTER BASIS	TMECC METHOD
TOTAL SOLIDS (%)	90.01	100.00	03.09-A
MOISTURE (%)	9.99	0.00	03.09-A
ORGANIC MATTER (%)	89.29	99.20	05.07-A
ASH (%)	0.72	0.80	05.07-A
SOLUBLE SALTS 1:5 (MMHOS/CM)	0.04	-	04-10-A
pH 1:5 (UNITS)	4.94	-	04-11-A
TOTAL NITROGEN (%)	0.279	0.310	04.02-D
ORGANIC NITROGEN (%)	0.278	0.309	CALC
AMMONIA NITROGEN (%)	0.0009	0.001	04.02-C
AMMONIA NITROGEN (PPM)	8.8	9.7	04.02-C
NITRATE NITROGEN (%)	0.0000	0.0000	04.02-B
NITRATE NITROGEN (PPM)	0.0	0.0	04.02-B
TOTAL PHOSPHORUS AS P (%)	0.010	0.011	04.03-A
TOTAL PHOSPHORUS AS P2O5 (%)	0.023	0.025	04.03-A
TOTAL POTASSIUM AS K (%)	0.021	0.023	04.04-A
TOTAL POTASSIUM AS K20 (%)	0.025	0.028	04.04-A
TOTAL CALCIUM (%)	0.142	0.157	04.05-Ca
TOTAL MAGNESIUM (%)	0.022	0.025	04.05-Mg
TOTAL COPPER (PPM)	1.4	1.5	04.06-Cu
TOTAL IRON (PPM)	154.8	172.0	04.05-Fe
TOTAL MANGANESE (PPM)	51.1	56.8	04.06-Mn
TOTAL ZINC (PPM)	16.9	18.8	04.06-Zn
SULFATE AS SO4 (PPM)	401.1	445.6	04.05-S
BORON (PPM)	23.8	26.4	04.05-B
SODIUM (%)	0.004	0.004	04.05-Na
CHLORIDE (%)	0.004	0.004	04.05-Cl
AG INDEX	45.2	45.2	CALC
C/N RATIO	169	169	CALC
AMMONIA-N/NITRATE-N RATIO	<1	<1	CALC

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TMECC = "TEST METHODS FOR THE EXAMINATION OF COMPOSTING AND COMPOST"; US COMPOSTING COUNCIL; AUG 2001; W.H. THOMPSON

ANALYSIS SUPERVISED BY

DATA APPROVED FOR RELEASE BY

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Colorado Analytical LABORATORIES, INC.

LABORATORY ANALYSIS REPORT

REPORT TO: JEFF RAVAGE

BILL TO: COALITION FOR THE UPPER SOUTH PLATTE
P.O. BOX 726
LAKE GEORGE CO 80827

LAB NO: 27296.01
DATE RCVD: 11/14/16
REPORTED: 11/30/16
PO NO.:

PROJECT: FUNGAL DEGRADATIONI STUDY

SAMPLE ID: **BERRYOW COMPOST**
MATRIX: COMPOST
SAMPLE DATE: 11/14/16

	AS RECEIVED BASIS	DRY MATTER BASIS	TMECC METHOD
TOTAL SOLIDS (%)	46.74	100.00	03.09-A
MOISTURE (%)	53.26	0.00	03.09-A
ORGANIC MATTER (%)	16.89	36.15	05.07-A
ASH (%)	29.84	63.85	05.07-A
SOLUBLE SALTS 1:5 (MMHOS/CM)	2.36	-	04-10-A
pH 1:5 (UNITS)	7.01	-	04-11-A
TOTAL NITROGEN (%)	0.264	0.566	04.02-D
ORGANIC NITROGEN (%)	0.262	0.561	CALC
AMMONIA NITROGEN (%)	0.0018	0.004	04.02-C
AMMONIA NITROGEN (PPM)	18.4	39.3	04.02-C
NITRATE NITROGEN (%)	0.0002	0.0004	04.02-B
NITRATE NITROGEN (PPM)	1.7	3.7	04.02-B
TOTAL PHOSPHORUS AS P (%)	0.027	0.058	04.03-A
TOTAL PHOSPHORUS AS P2O5 (%)	0.062	0.133	04.03-A
TOTAL POTASSIUM AS K (%)	0.061	0.130	04.04-A
TOTAL POTASSIUM AS K20 (%)	0.073	0.155	04.04-A
TOTAL CALCIUM (%)	3.600	7.703	04.05-Ca
TOTAL MAGNESIUM (%)	0.128	0.274	04.05-Mg
TOTAL COPPER (PPM)	4.3	9.3	04.06-Cu
TOTAL IRON (PPM)	2639.4	5647.5	04.05-Fe
TOTAL MANGANESE (PPM)	183.4	392.5	04.06-Mn
TOTAL ZINC (PPM)	29.9	64.0	04.06-Zn
SULFATE AS SO4 (PPM)	16,125.7	34,504.2	04.05-S
BORON (PPM)	22.6	48.4	04.05-B
SODIUM (%)	0.003	0.007	04.05-Na
CHLORIDE (%)	0.002	0.005	04.05-Cl
AG INDEX	67.2	67.2	CALC
C/N RATIO	34	34	CALC
AMMONIA-N/NITRATE-N RATIO	10.7	10.7	CALC

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