Carbon sequestration potentials of fungally produced composts

Experimental Design

Intent:
To monitor and measure the decay of a spectrum of white and brown rot mushrooms to determine post decay organic content and carbon form under ideal conditions.

Hypothesis:
Fungally produced composts should retain a high amount of organic material. This will be in the form of chitin from the fungal mass, cellulose, and lignin. White rotters, that digest lignin and leave cellulose behind, will have less carbon remaining in their compost than Brown rotters, which digest cellulose and leave behind stable lignin. Both will contain carbon in forms that potentially sequester the carbon for a meaningful amount of time. In carbon offset terms, that would mean 50-100 years of more. Both will have a lower C/N ratio than the controls, suggesting that composting woody debris using fungi will increase phytonutrient availability.

Design:
Each of the 10 strains will be cultivated in 16 standardized bags. These Standardized bags will consist of 4 k of substrate. 10 bags will be of a softwood- conifer based sawdust. Four bags will contain a mixture of hardwoods (Walnut, Cherry, Oak) And 2 bags, one hardwood and one softwood, will act as negative control. Inoculations will be by liquid culture so that the weight of each bag is consistent, controls will be inoculated only with sterile water of a volume equal to the liquid inoculant. This will give us a total of 160 bags for the experimental run. All bags will be steam pasteurized prior to inoculation.

Each strain will be supplied to The Sam Mitchell Fungarium at Denver Botanic Gardens for genetic bar-coding. This will be performed via DNA extraction, PCR, and Sanger sequencing of the nuclear ribosomal internal transcribed spacer region. If DNA sequencing of fungal communities/contaminated cultures is required, fungal metabarcoding using Illumina MiSeq will be performed. Each strain will be proof-fruited separately for positive identification of the teleomorphs, as is standard for our investigations.

The bags will be monitored monthly for weight and checked visually for contamination. Contaminated bags will be removed. Any strain with over 3 contaminated bags will be removed from the study and a new group will be recreated.

The bags will be prepared fully inflated so that fruiting may occur. The bags will be sealed with non-fusing techniques so that they may be opened when a flush of mushrooms is produced and the fruit harvested. Once a bag has completed a flush, it will be closed and placed in cool darkness for 30-60days. The process will be repeated 3 times (3 flushes) to simulate 3 seasons and to complete the digestion of the substrate. All fruits will be weighed, photographed with a portion retained as vouchers.

After the completion of the trial, all bags will be weighed for the final time and each bag will have a moisture test. Randomly, 50% of the bags will be designated for a standard compost test, and the other 50% will undergo Detergent Fiber Analysis to determine % of cellulosic and lignitic carbon. The control bags will be treated exactly like the strains they are associated with.

Analysis:

Weight change will be compared to moisture loss from beginning to end. Fruit removed will be subtracted from each bag for a final weight loss. The unaccounted for weight loss fraction will be assumed to be carbon dioxide loss via respiration. Carbon loss will be estimated as 12/44 of weight loss. This is the standard conversion for the molecular weight of a carbon atom versus a molecule of carbon dioxide. Each species will have all bags averaged to determine standard deviation and p values per species. One random bag from each species/substrate class will have an ergosterol assay performed on it to estimate the conversion of substrate to fungal biomass.

Each category- brown and white will be similarly averaged, as will softwood and hardwood substrates. We will then be able to examine each species performance against the baseline of a typical white or brown rotter.

The permanence value of the sequestered carbon will be established by the form that carbon takes (Cellulose or lignin) and established estimates of dwell time for each complex.

Species list:

White Rotters

Pleurotus ostreatus (Oyster mushroom) Strain WC-Post2019CUSP02

Trametes versicolor (turkeytail) Strain: WC-Tversi2019CUSP01

Flamulina velutipes (Velvet foot, Enoki) Strain: WC-Fvelu2020CUSP01

Stropharia rugossoannulata- (Wine Cap, wild type) Strain: CS-Srugg2022CUSP01

Griffola frondosa (Hen of the woods) Strain: CS-Gfron2022CUSP01

Brown Rotters:

Neolentinus lepideus (The Trainwrecker) Strain: CS-Nlepi2022CUSP01

Neolentinus ponderosus (Sawgill) Strain: CS-Npond2022CUSP01

Laeteoporus sulfureus Strain: (Chicken of the Woods) CS-Lsulf2022CUSP01

Fomitopsis officianalis (Agarakon) Strain: CS-FoffiCUSP01

Fomitopsis pinicola (Red belted polypore) Strain: WC-Fpino2020JStack01

WC- Wild Collected, CS- Commercial Strain