

Oh My! Mycelia!

By

Leuca Hanish

Department of Environmental Science

Allegheny College

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Dr. Beth Choate

Date

Dr. Adrienne Krone

Date

Pledge

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Abstract

Increases of atmospheric carbon dioxide through the industrial age of human activity on the Earth has, according to most scientists, caused an alarming rate of global warming resulting in major negative impacts on the environment. Nonetheless, there are still few solutions being developed to reduce this trend. One method of reducing atmospheric carbon dioxide, which has been largely ignored, is the promotion of fungal growth. Fungi have great potential to sequester immense amounts of carbon dioxide. In doing so, the fungi also play a large role in ecosystem restoration, a major necessary component in fighting climate change. Climate change has decreased fungal health globally; therefore, promoting fungal growth could be vital. For these reasons, the experiment outlined in this study is designed primarily to assess which natural forested environments are favorable for greater and faster fungal growth. The results show which tree types, native to western Pennsylvania, promote the most fungal growth when that tree species sheds woody material to the forest floor or when the tree dies and falls, providing fungi with their primary food source, wood. The soil and wood samples were placed in a controlled environment and served as a replication of the woody debris covering forest floor soil as a means to observe how fungi grow under those controlled conditions. With the results of this study, new forest management plans could be developed with the intention of increasing the total mass of fungi that would be capable of sequestering more carbon across the planet.

Introduction

Global warming, caused by human-induced increases in carbon dioxide concentrations in the atmosphere, is a broadly recognized environmental concern for the Earth (Chao & Feng, 2018, Thompson, 2010). Changes are necessary to reverse global warming to sustain life, in its current state, on the planet (Rhodes, 2019). Several detrimental effects have already been observed as a result of the changing climate such as more frequent and extreme forest fires, droughts, floods, and hurricanes (Abatzoglou & Williams, 2016, Chaudhary & Piracha, 2021, National Wildlife Foundation, 2019).

The biosphere has many systems that greatly contribute to carbon sequestration and reduction of global warming, not the least of which is fungi. Fungi have an immense capability to sequester carbon through removal of atmospheric carbon dioxide (Clemmensen et al., 2013). They are responsible for 80 percent of all carbon dioxide sequestration, not including the sequestration done by the ocean (Gray, 2021). Fungi are able to outcompete bacteria, fixing the carbon to the soil, whereas bacteria give off carbon dioxide (Averill et al., 2014). Unfortunately, agriculture, urbanization, and other human-induced destruction of ecosystems has had a negative impact on the health of the fungal community (Science Daily, 2019), reducing its ability to sequester carbon. The negative effect on fungi is predicted to worsen in the coming years (Větrovský et al., 2019, Averill et al., 2018). Climate change decreases health in some species of fungi more than others, causing fungi that are less adaptable to the effects of climate change to die off. This results in less diversity of beneficial fungi, and plant species that relied on these fungi are left with less support and nutrients (Millar & Bennett, 2016).

Fungi are a ubiquitous and critical resource, forming some of the largest organisms on Earth, including one specimen in Malheur National Forest in Oregon believed to be the world's largest covering 10km² (Haneef et al., 2017) and the world's oldest living organism believed to be approximately 8,650 years old (Zhang, 2017). Fungi, like animals and plants, belong to their own kingdom (Schwartzberg et al., 2019). A common misconception is that mushrooms are the dominant form of fungi; however, mushrooms are simply a miniscule surface expression, like a flower on a tree, of the mass underneath (Fungi Perfecti, 2021). In one square inch of soil, there is approximately eight miles worth of the underground portion of fungi, known as mycelia (Stamets, 2005). Under each footstep, the branches of mycelia, which transfer chemicals and nutrients using electrolytes, stretch for 300 miles (Schwartzberg et al., 2019). Mycelia form

important symbiotic connections, called mycorrhizae, with trees and other plants (Frank, 2004). This allows them to help trees adapt to climate change (Usman et al., 2021) by allowing trees to send signals and share nutrients amongst each other (Schwartzberg et al., 2019) and by providing nutrients to saplings after forests have burned down (Kariman et al., 2018). Through these relationships, mycelia are capable of acting as one of the planet's primary natural carbon dioxide sequestering agents (Clemmensen et al., 2013). Around 30 percent of the carbon dioxide plants absorb is stored in the mass of the plant itself; the other 70 percent is moved to the soil where it is stored within the cell walls of fungi, and in return, valuable nutrients are sent to the plant through its roots (Schwartzberg et al., 2019). Given that carbon dioxide is the greenhouse gas contributing the most to global warming (Reilly et al., 2020), mycelia are serving a critical role by sequestering carbon within the soil. Mycelia take carbon dioxide from the plants that absorb it from the atmosphere via photosynthesis and then store it in soil in the form of its own mass, allowing plants to continue absorbing more carbon dioxide (Gray, 2021). The branching mycelia move nutrients around and provide trees with what they need to survive (Behie & Bidochka, 2014). Without food, which for them is primarily woody materials, fungi will die off (Ibach, 2012) and can no longer complete their important roles.

Fungi are also capable of fertilizing soil, serving medicinal purposes, (Chang & Wasser, 2017), and acting as a building material (Haneef et al., 2017). Researchers have also found that enhanced growth of fungi could be key to ecosystem restoration (Nuñez et al., 2021, Science Daily, 2019, Chang & Wasser, 2017). Mycelia are not the only type of fungi that provide services such as ecosystem restoration; white rot fungi, for example, are capable of breaking down pollutants such as pesticides, polycyclic aromatic hydrocarbons, and polychlorinated aromatic compounds (Lang et al., 1997, Deshmukh et al., 2016). Past ecosystem restoration and carbon sequestration efforts have focused primarily on above ground plant growth, commonly ignoring the importance of what is happening underground (Nuñez et al., 2021). Researchers commonly have not recognized the important role that fungi play in support of thriving plant growth (Kozioł et al. 2018). Increasing the amount of fungi in soil could be vital for ecosystem restoration and carbon sequestration, and it could also help prevent future ecosystem degradation (Rashid et al., 2015). In an experiment, it was discovered that over a month's time, mushroom spawn introduced to heavy oil spilled in soil would result in almost all of the oil being consumed and large mushrooms being formed. The mushrooms then brought in flies, who attracted birds,

who brought in seeds, which in turn resulted in lush plant growth where the oil once saturated the soil (Stamets, 2000). This experiment was important in providing data about what certain fungi (in this case, Oyster mushrooms) can do. The Oyster mushrooms were observed to be able to degrade the oil in the soil, thriving under these toxic conditions. This is because fungi will decompose any substance containing hydrocarbons, making oil, wood, and other similarly composed materials perfect “food” for fungi (Schwartzberg et al., 2019). The experiment also showed that fungi are capable of restoring environments, and that its growth and cultivation can teach us more about fungi and what beneficial capabilities they have.

I conducted a study with the objective of developing an approach to grow the densest mass of fungi on multiple common species of wood native to western Pennsylvania. This work was done to understand what steps can be taken to enhance the rate of beneficial fungal growth to maximize its effect of carbon sequestration to help reduce carbon dioxide levels in the atmosphere, as well as maximize its effect of ecosystem restoration. Ectomycorrhizal (EcM) fungi, ericoid mycorrhizal (ERM) fungi, and arbuscular mycorrhizal (AM) fungi are the main types of fungi that form symbiotic relationships with plants. EcM fungi are the type of fungi the study will focus on growing because the other aforementioned types are microscopic (Gadd, 2013) and therefore more difficult to observe and measure. EcM, on the other hand, appears in visible mushrooms and visible underground networks of mycelia (Charya & Garg, 2019). EcM fungi have been shown to absorb 70 percent more carbon dioxide out of the atmosphere than AM fungi can (Averill et al., 2014). This is because they can consume more nitrogen in the soil, therefore taking it away from bacterial microorganisms who need the nitrogen to survive. When the fungi outcompete the bacterial microorganisms, those bacteria cannot thrive and break down organic matter in a process that would release much more carbon dioxide from the soil than the fungi (Averill et al., 2014).

For this study, wood from evergreen trees native to western Pennsylvania was collected for approximately half of the samples because they have been shown to form the strongest relationships with EcM fungi (Kariman et al., 2018).

The aim of this study was to determine the best tree material to provide food and promote the most fungal growth so that more information can be available for forest management practices. Those who decide which trees are cut down and which are left to stand in a forest used for lumber could prioritize leaving trees whose wood provides the best sustenance for fungi.

The question I will be answering is “How can I raise the densest crop of EcM fungi in the lab?”

Methods

Preparation for Substrate Collection:

In order to identify where samples should be collected, a map of Bousson was printed (ESS 440, 1996). In the lab, containers were labeled on the side and lid for identification in photographs. The number on each container corresponded with its sample sequence number on the spreadsheet (Table 1). Forty ounces of sand were autoclaved in an oven at 350 degrees Fahrenheit for 60 minutes, to be used in control samples. Before going into the field to collect samples, the materials listed in Appendix A: Materials were acquired. A bucket was filled with a water and dish soap mixture, and a spray bottle was filled with distilled water while another was filled with alcohol, all to be used in the decontamination procedure between each site.

Field Substrate Collection Procedure:

During the fall of 2022, I first traveled into the Bousson Forest (41.5978535, -80.0455057) and then Allegheny College's campus (41.6508332, -80.1417449) in Pennsylvania and collected wood from 9 individual trees of different varieties, making sure 4-5 of them were evergreen species. Wood from deciduous trees were included so that the difference between evergreen and deciduous trees could be evaluated. The variable in my experiment was the species of tree from which material was collected to serve as the carbon source in each sample. A map displaying tree groves throughout Bousson Forest was used to locate trees. When a tree of a desired type was located, I ensured that there were mostly other trees of the same species in the surrounding area. Trees crowded by other trees or located on significant slopes were not selected. As close to the base of the tree as possible, the surface of a flat portion of soil in a square of at least 3'x3' was raked to remove leaves and other litter (Figure 1). Pictures of the raked soil and the tree and surroundings were taken at each location.



Figure 1. 3'x3' raked soil at the sugar maple tree site, before soil was collected.

Using a hand shovel and wearing disposable gloves, a 1-gallon-sized Ziploc[®] bag was filled with soil from within the 3'x3' raked square. Soil was collected from the surface to approximately 6 inches down from up to 5 different points within the square, removing large rocks and pieces of leaves and other plant matter including roots. Using a Sharpie[®] marker, the bags of soil were labeled with a number (the first tree species location is 1, second tree species location is 2, etc.) and the tree type. Leaf or pine samples were collected in a separate 1-quart Ziploc[®] bag for later verification of the tree type. The bags were labeled with the tree number and the preliminary tree species identification. At each tree, small branches were broken off by hand or with pruning shears until they mostly filled a 1-gallon-sized Ziploc[®] bag. When branches were out of reach, bark was instead scraped off using a hatchet. Wood was not collected from the forest floor because this would add a variable regarding how long each piece of wood had already been decomposing. The Ziploc[®] bags containing small branches or bark were labeled

with the sampling location number and species of tree it came from. All collected wood and soil materials were placed into a bag separate from tools and other supplies. Using a scrub brush and the bucket filled with soapy water, the shovel, rake, and pruning shears and/or hatchet were cleaned. All the used tools were rinsed with alcohol using the spray bottle and then sprayed with distilled water. Disposable gloves were then removed and placed into a garbage bag. The clean tools were placed back into a clean Ziploc® bag and then into a bucket to carry. The lid on the soapy water bucket was snapped shut, and the bucket was carried to the next sampling location. Ideally, before departure, GPS location would be recorded and noted on a field map. Cell signal could not be found in Bousson Forest, so approximations were first made on the field map and then later confirmed by searching the area on Google Earth. These steps were repeated until materials were collected from 9 species of trees.

After 7 species of trees were visited, no trees could be found which could be confidently identified as new species that had not been sampled from yet. Therefore, all equipment was moved to the gravel track in the northeast corner (41.6508332, -80.1417449) of Allegheny College's campus, and the final trees, sycamore and locust, were sampled in the new location.

Experiment Sample Preparation Procedure:

All tools were washed with soapy water and rinsed with distilled water before use, and disposable gloves were worn to prevent contamination of samples. On a cutting board, each type of wood was finely chopped with pruning shears. The chopped wood was placed into a new Ziploc® bag and labeled with the tree species name and number. After chopping one wood type, the cutting board and pruning shears were cleaned with a water and dish soap solution in the sink then sprayed with distilled water from the spray bottle.

Sample containers were labeled numerically (Table 1). Each container was filled with 200g of soil and 15 grams of woody material. Lids were placed onto the samples, and three of the corners were snapped shut, leaving one corner open for airflow and for easy lid removal to facilitate inspecting, adding moisture, and photographing. After one tree species sample set had been prepared, those samples were placed away from the work area. New disposable nitrile gloves were changed into, the soil and woody substrate components from the next tree species were gathered, and the steps were repeated for the next five-container sample set. Used coffee

grounds were used as a control because they are commonly used by mushroom-growers and are known to be an efficient substrate for fungi (Krishnappa et al., 2022). If there were no fungi in these samples, that would have served as an indicator of a problem with the experiment. Sand was also used as a control because the samples with sand should not have grown fungi, so any fungal growth would have indicated an environmental factor that was not accounted for. All conditions and maintenance were kept as fixed as possible between samples. Coffee grounds were collected out of the compost bin in the student-run coffee shop, GFC, at Allegheny College. Disposable gloves were used to fill a gallon Ziploc® bag. A set of five containers were each filled with 200g of coffee grounds. Another set of five containers were each filled with 200g of previously autoclaved sand.

Each sample was then photographed for later comparisons. One photograph was taken directly over the sample, and one was taken directly from the side, ensuring the sample number was visible in the photo. Consistency was achieved by selecting a uniform place, on the lids or the container sides, to line up with the edges of the viewfinder in the camera. Each sample was moistened with distilled water, initially with five pumps from the spray bottle. In an empty sample container, the weight of the amount of water given to the samples was measured. The five pumps of the spray bottle weighed 16g.

Table 1. Sample number and tree species for each sample, as well as the type of wood collected from each tree.

Sample #	Tree/substrate	Small branches or bark
1-5	Red pine	Small branches
6-10	White pine	Small branches
11-15	Sugar maple	Small branches
16-20	Scotch pine	Small branches
21-25	Oak	Small branches
26-30	Norway spruce	Bark
31-35	Hemlock	Small branches
36-40	Sycamore	Bark
41-45	Locust	Bark
46-50	Coffee	
51-55	Sand	

Note. Each tree species sample, the coffee grounds samples, and the sand samples (numbered 1-55) are noted on the above chart to identify what was placed in each sample.

Each wood type was finely chopped and placed on top of soil. Coffee grounds and sand were by themselves, without soil or wood, for a 5-container set each. Eleven different sample groups all contained in a quintupled container set resulted in 55 individual containers. Each tree species was numbered in the order in which they were visited for substrate collection. Samples were stored in closed cabinets in Carr Hall at Allegheny College, Meadville, PA for approximately 2 months.

Experimental Observations, Maintenance, and Record-Keeping:

Disposable nitrile gloves and an N-95 or KN-95 mask was worn at all times when handling and checking samples. The samples were watered as needed using a spray bottle with distilled water and sprayed until damp. Bottled distilled water was used to ensure that the water I used did not contain contaminants that could alter the growth of the fungi. The volume and number of sprays of water added to the samples were recorded each time. At least 3 times during the experiment, the temperature in the room at a location close to the samples was measured and recorded. After approximately 1 month, and again after 2 months, samples were photographed. Photographs were taken of the top and side of each sample at a consistent distance by lining up the edges of the containers at a consistent point on the viewfinder. The number label on the lid was in view in each photograph. Each sample was placed in the same location and with the same lights on for photographs. Photos were stored in a Google Drive folder and sorted by date and sample number. If any plants grew in the samples, they were removed so that roots would not be mistaken for mycelia. No two samples were ever open at the same time, and the lids were placed on each sample before they were returned to storage to limit the potential for cross contamination.

Table 2. Log of water added to all samples containing soil and wood.

Date	Number of sprays	Weight of water added (g)
11/3/22	5	16
11/9/22	7	22
11/16/22	7	22
12/21/22	5	16

Note. With the spray bottle, 1 spray = ~3.2g. The coffee grounds were more damp than the soil and wood upon collection, so the samples with coffee grounds were only given 5 sprays when others were given 7 and then not watered on 12/21 to avoid oversaturation. The samples with sand did not absorb water, and there was no significant evaporation, so they were watered on only the first 2 dates listed above and given 5 sprays each time.

Leaf and needle samples collected on site, along with the photographs taken of the trees (see above section “Field Substrate Collection Procedure”), were used to confirm that the trees were identified correctly on site. A tree identification guidebook was followed to identify the trees using their needles and leaves (Tekiela, 2004).

Soil type present at each sampling location was determined using an online soil survey (USDA, 2019). An area of interest was manually selected surrounding the sampling locations, resulting in a map identifying the soil types within the areas (Figures 6 and 7), along with descriptions of each type (Appendix D). The soil types on this map were then lined up with the locations of the sampling locations as marked on Google Earth (Figures 2 and 3) to decide which soil type was present at each location, and therefore decipher which soil types were present in each sample group (Table 5).

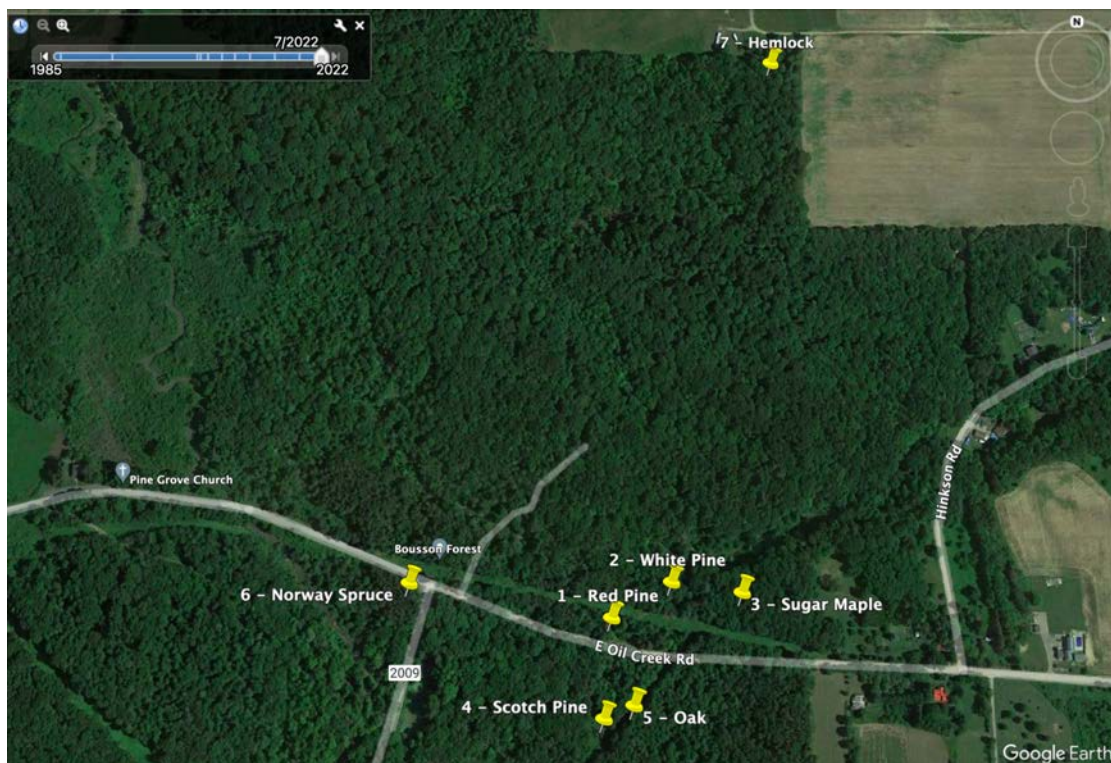


Figure 2. Sampling locations in Bousson Forest pinned and labeled with number and tree species on Google Earth.

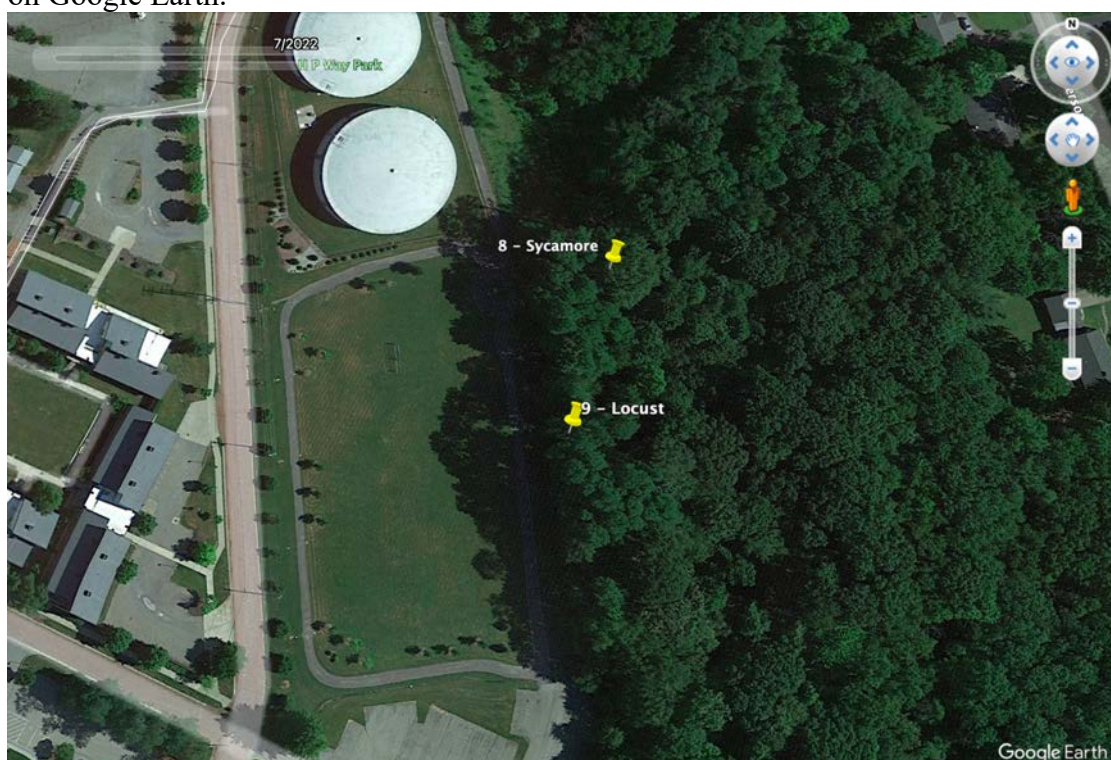


Figure 3. Sampling locations at Allegheny College pinned and labeled with number and tree species on Google Earth.

Data Analysis:

Using the program Red Line Tools, a grid was overlaid onto the photographs of the first 3 samples in each sample group at the end of the 2 month observation period. The surface area was estimated using point counting methods (Neilson & Brockman, 1977) on the overhead view of each container. Locations where the lines of the grid intersected each other were treated as singular points, and it was then noted whether fungi were present or not at those particular points. The number of points where fungi were counted as present were divided by the total number of points counted, which was multiplied by 100 to yield the percentage of the surface area covered by fungi. The number of points needed to achieve accurate results was recommended by one source to be 1000 or more (Neilson & Brockman, 1977) and by another source to be 300-500 (Petrog, n.d.). The number of points on each sample in this experiment ranged from 1015 to 1296.

After all data was collected, I compared the calculated percentage of surface area covered by visible fungi (as a measure of the relative degree of fungal growth) of each tree species included in the experiment. The averages and standard errors were calculated for each sample group (Table 3).

Results

Fungal growth was visible on 50 of the 55 samples, with sand being the only substrate resulting in no fungal growth. The sample group containing woody substrate which produced the highest average percentage of surface area covered by fungi, 57.25%, was the sugar maple group, or sample numbers 11-13 (Table 3). The next sample group, containing oak wood and numbered 21-23, contained less than half of the amount of fungi as the sugar maple group (19.5% covered, Table 3). For all other groups, the percentage of the sample surface covered in fungal growth was minimal. The red pine, white pine, Scotch pine, and hemlock groups all had average percentages of surface area covered by fungi between 1 and 3 percent. The Norway spruce, sycamore, and locust groups all resulted in percentages below 1 percent (Table 3).

The samples with coffee grounds produced the highest percentage of surface area covered by fungi; they contained an almost complete coverage, with an average percentage of 98.8%. The other control samples, which contained sand, produced no fungi.

The samples containing sugar maple wood were the first samples that had fungal growth. The samples were assembled and watered on November 3, and by November 9 there was significant visible mycelial growth on the sugar maple samples (Figure 4). Much smaller amounts of fungi were visible on the oak and locust samples, and no fungi were apparent on any of the other samples by this time. By November 28, all samples which previously contained fungi had an increase in amount, and all samples showed some fungal growth with the exception of Norway spruce and sycamore. The Scotch pine samples had visible mycelial growth by the 28th, but by December 5, all 5 samples were contaminated with small white insects and almost all fungi were no longer present. On the final day the samples were examined, January 7, the insects remained but there was also a resurgence of fungi, returning approximately to the same amount that were present previously.



Figure 4. Images of sample 12 (sugar maple) over time.
Left to right: November 9, 2022, November 28, 2022, January 7, 2023.

Note. Within the first week, the fungi in sample 12 were present in approximately the same amount as at the end of the experiment. The primary type of fungi changed from a webby mycelia to a pin mold during the second month. An adjustment in the primary type of fungi was common in the sugar maple samples, but no other samples.

The samples with coffee grounds were slow to show the first signs of fungal growth, but by the end of the experiment were in the lead with approximately 99 percent surface area covered by fungi. Not represented in the results is that the side views of the coffee ground containers each had as much fungi as the aerial view, while none of the soil and woody substrate samples showed signs of fungi growing vertically downward into the soil.

Each sample group displayed consistency among samples, as percentages were relatively similar from one sample to the next, and there were no outliers.

Bark, rather than small branches, were collected from the Norway spruce, sycamore, and locust trees due to an absence of tree branches within reach from the ground. A trend was found that the samples with bark had a lower relative amount of fungi than the samples with small branches.

Samples with soil and woody materials, though watered equally, differed from each other in apparent moisture content.

Table 3. Table of percentage of surface area covered by fungi in each sample, along with averages and standard errors for each sample group.

Tree species/substrate	Sample #	% Surface area with fungi	Average %	Standard Error
Red pine	1	0.47	2.54	1.038
	2	3.45		
	4	3.7		
White pine	6	2.61	1.6167	0.612
	7	0.5		
	8	1.74		
Sugar maple	11	43.1	57.2533	7.088
	12	63.64		
	13	65.02		
Scotch pine	16	1.47	1.1167	0.228
	17	1.19		
	18	0.69		
Oak	21	20.03	19.5033	4.926
	22	10.72		
	23	27.76		
Norway spruce	26	0.57	0.5567	0.013
	27	0.57		
	28	0.53		
Hemlock	31	1.55	1.567	0.165
	32	1.29		
	33	1.86		
Sycamore	36	0.08	0.213	0.085
	37	0.19		
	38	0.37		
Locust	41	0.23	0.32	0.071
	42	0.27		
	43	0.46		
Coffee	46	99.39	98.7967	0.312
	47	98.67		
	48	98.33		
Sand	51	0	0	0.000
	52	0		
	53	0		

Note. Each sample group contained 5 individual samples, and in each group, the first 3 in the numbered sequence were chosen for surface area calculations.

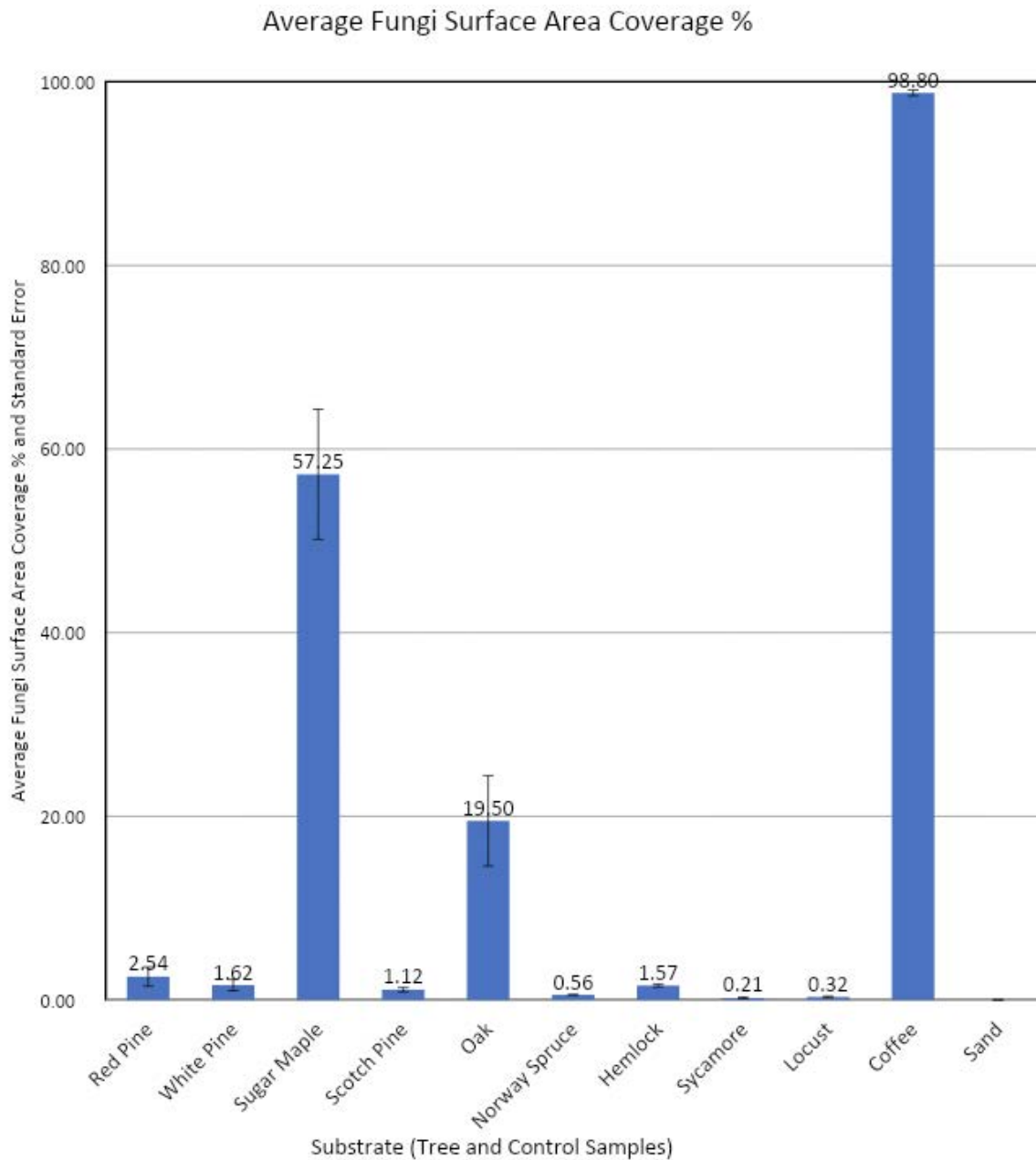


Figure 5. Bar chart of percentage of average surface area covered by fungi in each sample group, along with standard error bars.

Seventeen distinct types of fungi grew on the samples, with multiple types of fungi on many of the samples (Table 4). The samples used to determine the percentage of surface area covered by fungi (the first 3 out of the 5 in each sample group) were the same samples used to create table 4.

Table 4. Descriptions of fungi grown, along with which samples each type appeared in.

Type of fungi	Description	Samples containing type	Comments
Fungi A	White, webbed	Red pine, Scotch pine, hemlock	Mycelia
Fungi B	White, thin sheets	White pine, sugar maple, oak	
Fungi C	White, fuzzy, solid mass	Sugar maple, Scotch pine, sycamore, locust	
Fungi D	Green/gray/white, tiny and round, typically spread out and low in occurrence	Sugar maple	
Fungi E	White, tiny pins, look like dandelions	Red pine, white pine, sugar maple, Scotch pine, oak	Pin mold
Fungi F	Gray/dark gray, fuzzy, round, small, and packed together	Sugar maple	Lasiosphaeria spermoides (of sac fungi group)
Fungi G	Gray, thin-stranded webs, often reaches from one stick to another	Sugar maple, oak	
Fungi H	Gray, fuzzy, tiny pins sticking out from wood, thinner and more dense than fungi E	Sugar maple	Pin mold
Fungi I	Gray/brown, short hairlike strands	Sugar maple, Scotch pine	
Fungi J	Black, long strands, sometimes reaching from one side of a container to another	White pine	
Fungi K	Dense fuzzy green or white, maybe with some concentrated areas orange in color	White pine, hemlock, locust, coffee	Penicillium or trichoderma
Fungi L	Yellow or orange, perfectly round and smooth	White pine, sycamore	
Fungi M	White and red, slimy mass	White pine	
Fungi N	Black, tarlike or fuzzy, warpping around entire stick	White pine	
Fungi O	Green, webbed/hairlike	Oak	
Fungi P	Brown, tiny, slimy, oval-shaped masses	Oak, Norway spruce	
Fungi Q	White/brown, tiny, dog bone shaped	Coffee	

Note. A northeast North America field guide was used for identification (Barron, 1999).

Fungi C and E were the most common, found in samples within 5 of the 9 total sample groups. Fungi K was found in 4 of the sample groups, while fungi A and B were found in 3 groups. Fungi types which were unique to 1 sample group only were D, F, H (all in sugar maple), J, M, N (in white pine), O (in oak) and Q (in coffee).

With the exception of the sample group containing sugar maple wood, samples which had visible fungal growth early on remained consistent in the primary types of fungi present. All sugar maple samples contained mostly fungi A and G in the first month of the experiment, and in the second month of the experiment they diversified to contain 8 different types of fungi, with the most prevalent types being fungi E and F.



Figure 6. Close up images showing the common types of fungi grown in samples. Top left image: top, middle, then bottom right sticks, respectively: fungi F, fungi C, fungi E, on sugar maple. Top right image: fungi K in upper left and fungi Q in lower right, on coffee. Bottom left image: fungi E on all sticks and fungi B on middle and lower right sticks, on oak. Lower right image: fungi G on sugar maple (Table 4).

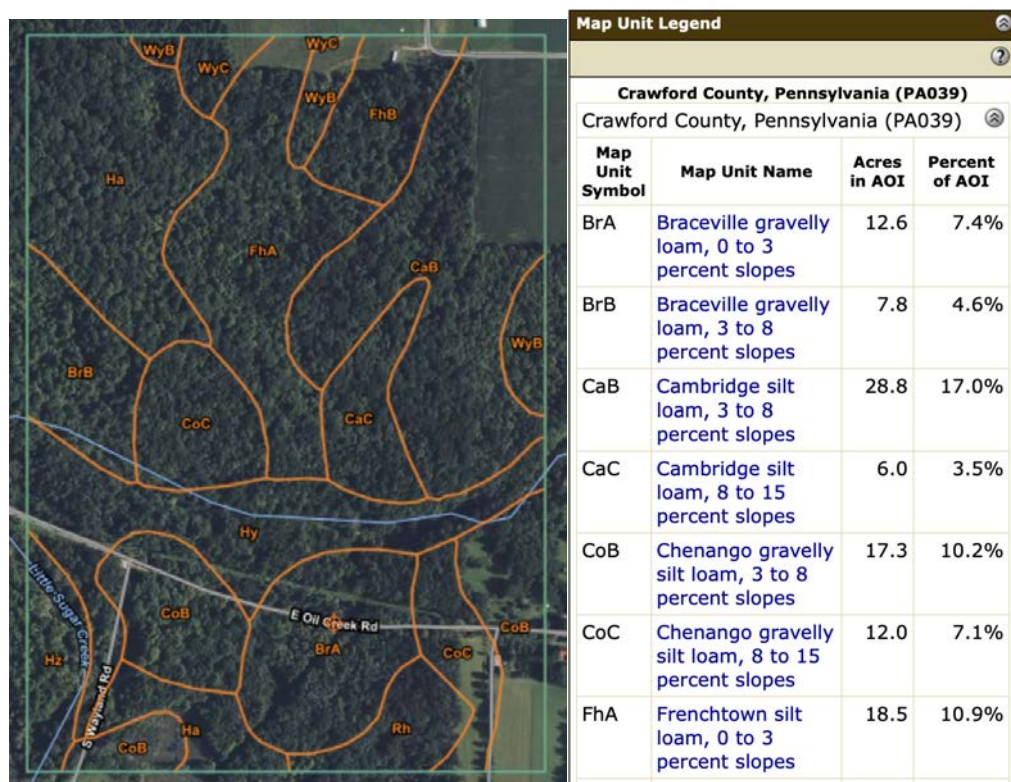


Figure 7. Soil type map covering sampling locations within Bousson Forest.



Figure 8. Soil type map covering sampling locations near the gravel track in the northeast corner of Allegheny College's campus.

Note. See appendix D for more thorough descriptions of each soil type, provided by the online survey (USDA, 2019).

Table 5. Soil types at each sampling location, along with percent slope of the ground and whether the tree was located in Bousson Forest or near the gravel track at Allegheny College.

Tree species	Soil type	Soil abbreviation	Percent slope	Sampling location
1. Red pine	Braceville gravelly loam	BrA	0-3	Bousson Forest
2. While pine	Braceville gravelly loam	BrA	0-3	Bousson Forest
3. Sugar maple	Braceville gravelly loam	BrA	0-3	Bousson Forest
4. Scotch pine	Braceville gravelly loam	BrA	0-3	Bousson Forest
5. Oak	Braceville gravelly loam	BrA	0-3	Bousson Forest
6. Norway spruce	Chenango gravelly silt loam	CoB	3-8	Bousson Forest
7. Hemlock	Frenchtown silt loam	FhB	3-8	Bousson Forest
8. Sycamore	Cambridge silt loam	CaB	3-8	Allegheny College
9. Locust	Cambridge silt loam	CaB	3-8	Allegheny College

Note. The red pine tree at the first sampling location was determined to be on Braceville gravelly loam soil. However, it closely bordered an area composed of Holly silt loam. Similarly, the Hemlock tree at the seventh sampling location was on Frenchtown silt loam, but was closely bordering Cambridge silt loam.

All samples from the Braceville gravelly loam soil type from an area with less than a 3 percent slope contained above 1 percent of surface area covered by fungi, including the samples with sugar maple wood, which had the most fungal growth (Table 3). Samples containing hemlock wood were located on Frenchtown silt loam soil but closely bordered the Braceville gravelly loam, and those samples also contained above 1 percent of surface area covered by

fungi. The samples not on or bordering Braceville gravelly loam soil, and on a 3-8 percent slope, contained less than 1 percent.

The moisture level of the soil upon collection from each sampling location had no correlation with the results. The soil at the sampling location of the sugar maple, which had the most fungal growth, was noted as being dry. The soil in the white pine, scotch pine, Norway spruce, and locust samples were also relatively dry. Soil which initially had a higher relative moisture content was collected at the red pine, oak, hemlock and sycamore sampling locations.

Discussion

My findings are most useful in temperate and boreal forested regions due to the presence of the tree varieties I tested. If samples from one variety of tree consistently produce the most fungi, this might mean that forest managers should not harvest these trees and should let them remain in forests after they die. Sugar maple and oak wood were shown to produce the most fungi, meaning these trees would improve the health and abundance of fungi in forests. Because these wood types resulted in a higher fungal mass, they are likely the best food for fungi. If these trees are targeted for lumber, or removed from forests after they die, fungi will be left with a lower quality of sustenance. As a result, there could be less fungi available to form symbiotic relationships with trees, meaning forests would have a lower capacity to absorb carbon dioxide from the atmosphere and store it in the ground.

The samples contained soil from directly underneath a tree, and wood from the tree was layered on top. The soil taken from the base of the tree already contained the mycorrhizal fungi which have formed a relationship with the tree species it was surrounded by; therefore, tree groves were targeted in this experiment over standalone trees to ensure the fungi could not be associated with a nearby species of tree. Once the branches were taken from the tree, they decomposed, serving as food for the fungi. This increased food supply results in healthier fungi which will then increase in mass. Mycorrhizal fungi act as decomposers alongside their standard jobs (Talbot et al., 2008), so the fungi collected in the soil will likely be the same fungi that remain in the samples to decompose the woody material.

All samples containing woody material were watered the same amounts at the same times (Table 2). Despite this, some samples appeared consistently more dry than others. The relatively dry samples contained less fungi by the end of the experiment, indicating that moisture levels impacted the results. Upon collection in the field, the more alive branches of the sugar maple tree were flexible and likely had a higher starting moisture content than the more brittle branches from the other trees. This moisture could have had an effect on the initial speed of fungal growth on the woody substrate. The samples with sugar maple branches had a strikingly high amount of fungal growth compared to all other samples.

Carbon, which is what fungi need as food to grow and survive (Schwartzberg et al., 2019), is primarily transported by sucrose, which is found in the phloem of all tree species.

Sucrose is the most mobile, though trees contain other carbohydrates which serve as carbon transport agents, such as glucose and fructose (Dominguez & Niittylä, 2021, Magel, et al., 2000). Maple trees have been shown in a study to contain more sucrose-rich sugar, as well as more sugar overall, than birch and hornbeam species. The maple sap used in the study contained over 3% sucrose, while birch sap contained less than 1% sucrose, and hornbeam had minimal amounts. The study did not compare sucrose content of more tree species, but it can be inferred that maple trees are proportionately rich in sucrose (Łuczaj et al., 2014). Oak trees have also been shown to have a relatively high sugar content, with much of that being sucrose (Gersony et al., 2021, Jolly, 2019). The sugar content, and specifically sucrose concentrations, in maple and oak trees may have allowed these tree types to support a higher mass of fungi than other observed species.

Validity of Results:

The control samples, 5 with used coffee grounds and 5 with sand, were used to assess the validity of results. Coffee is a common substrate among mushroom-growers, meaning the samples with coffee grounds, kept under proper conditions, should have contained the largest, or close to the largest, percentage of fungi over the surface area. Sand was used because it contains no materials fungi would feed on; therefore, fungal growth in the samples with sand would have pointed to a problem with the procedures. At the end of the observation period, the samples with coffee grounds had the most relative amount of fungi, while the samples with sand had none. This goes to show that the samples were kept in the proper conditions with no significant contamination.

Consistency between samples grouped by tree species indicates validity of results. Within each sample group, the largest standard error calculated from the averages of surface area covered by fungi was 7.1. With the exception of the three woody samples with the highest fungal yield, the standard errors were all below 1 (Table 3 & Figure 5).

Uncertainty and Limitations:

Pine and spruce trees have been shown in prior studies to foster more mycelial networks and other fungal growth (Kariman et al., 2018). In this study, the samples with pine or spruce wood yielded relatively low amounts of fungi compared to the samples with sugar maple and oak wood, both deciduous trees. No clear explanation has been found for why the evergreen species did not produce higher quantities of fungi.

Photographs of the samples were utilized for the point count method (see “Data Analysis” in methods section). The photographs contained some highlights and shadows which caused uncertainty when determining which points were coated in fungi. The samples were not discarded and therefore were available for reference when needed. For the purpose of this project, a couple hundredths or even tenths of percentage points of uncertainty do not significantly affect the results.

Future Studies:

Future studies could focus on truly isolating each sample type in separate cabinets to further reduce the potential of spores becoming airborne and “cross pollinating” other samples. The first of the samples with coffee grounds contains mycelia which are naturally colored green. Other coffee ground samples contain either orange or white mycelia. This difference in color could have been impacted by the different types of fungal spores that exist in the air and can latch on to anything hydrocarbon based (Schwartzberg et al., 2019). Differences in textures of fungal growth on the samples is also present, with some appearing to be textured like fur, spikes, sheets, webs, and more. Isolated samples in a future study could provide an answer as to whether the samples were cross-contaminated. Airborne fungal spores are ubiquitous (Schwartzberg et al., 2019), so they may have led to differences in types of fungi.

An additional beneficial approach could have been to add a confirmed sterilized control sample consisting of coffee grounds (which is the one media that is both a granular material to hold moisture and a carbon-rich substrate upon which to grow fungi) or other potential media to better understand the role that later introduced airborne fungal spores may play in my experiment (as opposed to spores already present in the sample of soil and wood collected).

Pine needles and leaves were collected in Ziploc bags, for later confirmation of tree type, and left undisturbed in a garbage bag in a closed cabinet for approximately 5 weeks. The needles collected from the Scotch pine, after the 5 weeks passed, were coated in thick layers of mycelia; at that point in the experiment, this bag contained more fungi than any of the samples with soil and wood, or the samples with coffee grounds. This observation indicates that pine needles could be a better substrate than the woody material used in the samples. Future studies could focus on pine needles rather than woody material and attempt to answer why the Scotch pine drastically differed from the other pine needles. The needles from the Scotch pine were green in color; the moisture content of the needles could have been key in catalyzing fungal growth.

The temperature of the samples was not specifically controlled but was maintained at the room temperature of the storage location in Carr Hall. Future studies could be conducted under specific controlled temperatures to see how temperatures may influence fungal growth rates and amounts.

The experiment ran for approximately 2 months, so it is unknown whether results would change if the samples were observed for a longer time. Future studies could set an observation period of more than 2 months to understand how the samples act over a longer time.

To reduce the impact of highlights and shadows in photographs of the samples, future studies could use finely ground wood that is able to be spread evenly on top of the soil. As an alternative to using photographs, sample containers that facilitate observation with a microscope could be beneficial in the point-count procedure and in the identification of types of fungi. Microscope slides could be placed on a mechanical stage that moves in even increments.

In this experiment, the woody substrates and soil within samples were not lifted to examine fungal growth beneath the surface. A procedure for observing and quantifying fungal growth within an entire sample would provide more information than a surface area observation.

Arbuscular mycorrhizal (AM) fungi, fungi not studied in this experiment because they are microscopic, are essential to ecosystems because they can support trees in soils with less organic materials than ectomycorrhizal (EcM) fungi can. AM fungi are predicted to become more dominant as soils undergo a rise in pH due to acid rain (Ohio University, 2020). Almost all tree species form connections with both AM and EcM fungi (Heklau et al., 2021), so a future study aiming to focus on AM fungi could use the same tree species as in this study. Findings

from a future study with a focus on AM fungi could lead to new implications for forest management practices.

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Appendix

Appendix A. Materials

Field - Collection Related Equipment and Materials:

- Distilled water
- Isopropyl Cleaning alcohol
- 2 spray bottles
- Pruning shears
- Disposable nitrile gloves
- 1000 grams of Quikrete All Purpose Sand
- Hand shovel
- 2 x 5-gallon clean decontamination buckets with lids for transportation - One for soapy wash water and one for equipment
- Dish soap
- Scrub brush
- 1-Gallon Ziploc® bags
- 1-Quart Ziploc® bags
- Sharpie® marker
- Tree identification guide
- Cellular phone with working camera and GPS location
- Field soil sieves (if the soil is rocky)
- Rake
- 2 large backpacks or buckets for carrying supplies and samples
- Tape measure
- Garbage bags
- Field notebook and pen for taking notes
- Hatchet

Laboratory - Sample Preparation Related Equipment and Materials:

- A minimum of 56 Placon EV1-16 Ecostar Recycled PET containers, 16 fluid ounces, 4.75" x 4.75" x 2.625" along with 56 compatible transparent lids
- Kitchen scale
- Thermometer
- Soil moisture meter
- Cutting board
- Knife
- Any other tool helpful in sizing woody material to sizes similar across soil and wood substrates
- Sieves for soil identification via grain-size distribution

Appendix B. Sample Images



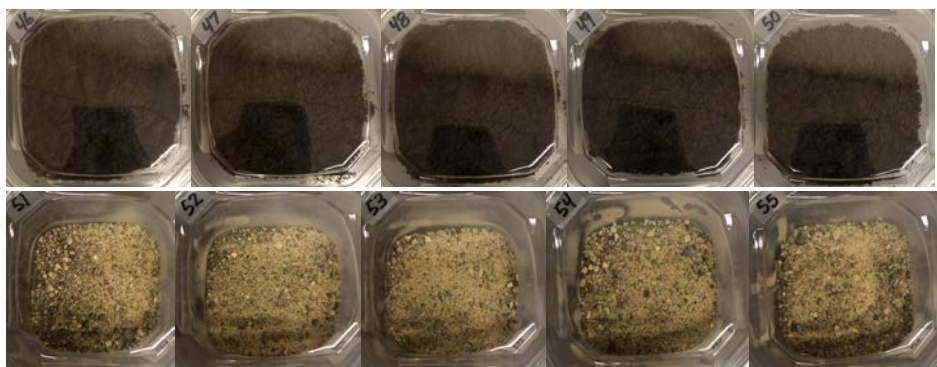


Figure 9. Initial photos of all samples, taken November 3, 2022.



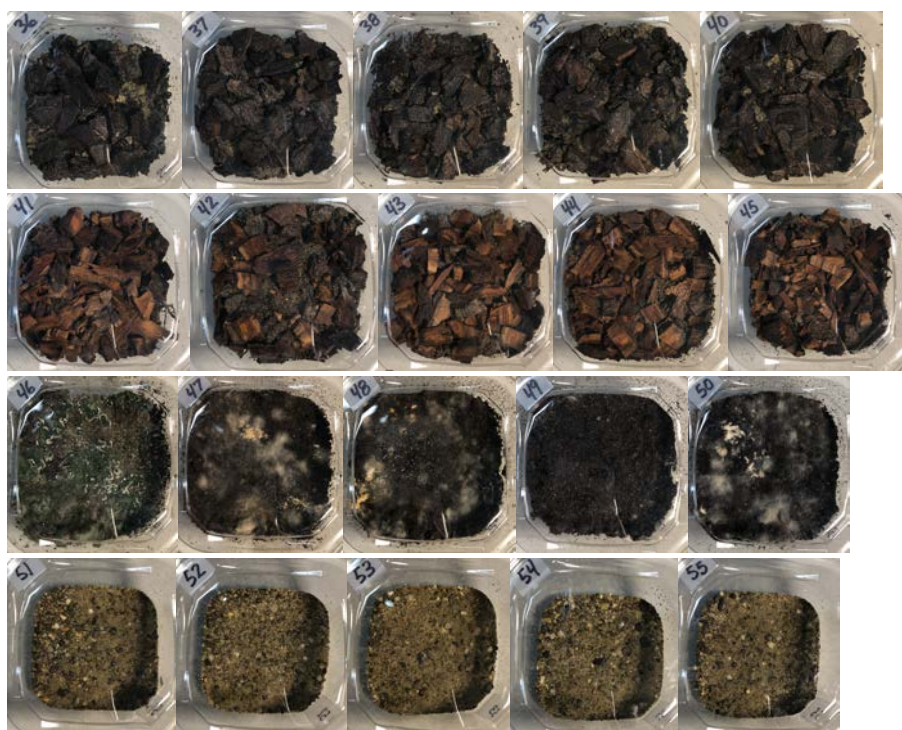


Figure 10. Final photos of all samples, taken January 7, 2023.

Appendix C. Tree images at each of the 9 sampling locations



Left to right, top row: red pine, white pine, sugar maple. Middle row: Scotch pine, oak, Norway spruce. Bottom row: hemlock, sycamore, locust.

Appendix D. Soil survey data: descriptions of soil types present at each sampling location

Description of Braceville

Setting

Landform: Outwash terraces
Landform position (two-dimensional): Toeslope
Landform position (three-dimensional): Tread
Down-slope shape: Linear
Across-slope shape: Linear
Parent material: Coarse-loamy outwash

Typical profile

Ap - 0 to 10 inches: gravelly loam
Bw - 10 to 22 inches: gravelly silt loam
Bx - 22 to 33 inches: gravelly silt loam
C - 33 to 60 inches: stratified sand and gravel

Properties and qualities

Slope: 0 to 3 percent
Depth to restrictive feature: 18 to 26 inches to fragipan
Drainage class: Moderately well drained
Runoff class: Low
Capacity of the most limiting layer to transmit water (Ksat):
Moderately low to moderately high (0.06 to 0.60 in/hr)
Depth to water table: About 16 to 25 inches
Frequency of flooding: None
Frequency of ponding: None
Available water supply, 0 to 60 inches: Very low (about 2.2 inches)

Interpretive groups

Land capability classification (irrigated): None specified
Land capability classification (nonirrigated): 2w
Hydrologic Soil Group: C/D
Ecological site: F139XY004OH - Moist Acidic Slopes
Hydric soil rating: No

Description of Frenchtown

Setting

Landform: Depressions
Landform position (two-dimensional): Toeslope
Landform position (three-dimensional): Base slope
Down-slope shape: Concave
Across-slope shape: Concave
Parent material: Till

Typical profile

Ap - 0 to 8 inches: silt loam
BEg - 8 to 13 inches: silt loam
Btg - 13 to 22 inches: silt loam
Btx - 22 to 50 inches: loam
BC - 50 to 56 inches: loam
C - 56 to 70 inches: loam

Properties and qualities

Slope: 3 to 8 percent
Depth to restrictive feature: 18 to 38 inches to fragipan
Drainage class: Poorly drained
Capacity of the most limiting layer to transmit water (Ksat): Very low
to moderately low (0.00 to 0.14 in/hr)
Depth to water table: About 0 to 10 inches
Frequency of flooding: None
Frequency of ponding: Frequent
Calcium carbonate, maximum content: 10 percent
Available water supply, 0 to 60 inches: Low (about 4.6 inches)

Interpretive groups

Land capability classification (irrigated): None specified
Land capability classification (nonirrigated): 4w
Hydrologic Soil Group: D
Ecological site: F139XY012OH - Wet Acidic Depression
Hydric soil rating: Yes

Description of Cambridge

Setting

Landform: Moraines, till plains
Landform position (two-dimensional): Summit, shoulder
Landform position (three-dimensional): Interfluve, side slope
Down-slope shape: Convex
Across-slope shape: Convex
Parent material: Till

Typical profile

Ap - 0 to 8 inches: silt loam
Bw - 8 to 24 inches: silt loam
Btx - 24 to 53 inches: loam
C - 53 to 72 inches: loam

Properties and qualities

Slope: 3 to 8 percent
Depth to restrictive feature: 18 to 30 inches to fragipan
Drainage class: Moderately well drained
Capacity of the most limiting layer to transmit water (Ksat): Very low
to moderately low (0.00 to 0.14 in/hr)
Depth to water table: About 18 to 24 inches
Frequency of flooding: None
Frequency of ponding: None
Calcium carbonate, maximum content: 10 percent
Maximum salinity: Nonsaline (0.0 to 1.9 mmhos/cm)
Available water supply, 0 to 60 inches: Low (about 4.9 inches)

Interpretive groups

Land capability classification (irrigated): None specified
Land capability classification (nonirrigated): 2e
Hydrologic Soil Group: D
Ecological site: F139XY004OH - Moist Acidic Slopes
Hydric soil rating: No

Description of Holly

Setting

Landform: Depressions on flood plains
Landform position (two-dimensional): Toeslope
Landform position (three-dimensional): Base slope
Down-slope shape: Concave
Across-slope shape: Linear
Parent material: Recent loamy alluvium

Typical profile

Ap - 0 to 10 inches: silt loam
Bg - 10 to 30 inches: silt loam
BC - 30 to 60 inches: loam
C - 60 to 80 inches: stratified gravelly sand to silt loam

Properties and qualities

Slope: 0 to 3 percent
Depth to restrictive feature: More than 80 inches
Drainage class: Poorly drained
Runoff class: Very high
Capacity of the most limiting layer to transmit water (Ksat):
Moderately high to high (0.20 to 2.00 in/hr)
Depth to water table: About 0 to 6 inches
Frequency of flooding: Frequent, None
Frequency of ponding: None
Available water supply, 0 to 60 inches: High (about 10.5 inches)

Interpretive groups

Land capability classification (irrigated): None specified
Land capability classification (nonirrigated): 3w
Hydrologic Soil Group: B/D
Ecological site: F139XY009OH - Wet Floodplain
Hydric soil rating: Yes

Description of Chenango

Setting

Landform: Kame terraces, outwash terraces
Landform position (two-dimensional): Backslope, toeslope
Landform position (three-dimensional): Tread
Down-slope shape: Convex, linear
Across-slope shape: Convex, linear
Parent material: Gravelly outwash

Typical profile

Ap - 0 to 10 inches: gravelly silt loam
Bw - 10 to 30 inches: very gravelly loam
C - 30 to 80 inches: extremely gravelly coarse sand

Properties and qualities

Slope: 3 to 8 percent
Depth to restrictive feature: More than 80 inches
Drainage class: Well drained
Runoff class: Medium
Capacity of the most limiting layer to transmit water (Ksat):
Moderately high to high (0.60 to 6.00 in/hr)
Depth to water table: More than 80 inches
Frequency of flooding: None
Frequency of ponding: None
Available water supply, 0 to 60 inches: Low (about 4.3 inches)

Interpretive groups

Land capability classification (irrigated): None specified
Land capability classification (nonirrigated): 2s
Hydrologic Soil Group: A
Ecological site: F139XY005OH - Dry Acidic Till Plains, F124XY009OH -
Coarse Terrace and Plain, F139XY006OH - Moist Till Highlands
Hydric soil rating: No

Note. Above soil data was taken directly from USDA web soil survey interactive map (USDA, 2019).

Appendix E. All close up images showing different types of fungi in samples



Left to right: fungi A on red pine, fungi B on white pine, fungi M and L on white pine



Left to right: fungi N on white pine, fungi J on white pine, fungi K on white pine



Left to right: fungi E and F on sugar maple, fungi E, C and F on sugar maple, fungi G on sugar maple



Left to right: fungi F and I on sugar maple, fungi B and E on oak, fungi O and E on oak



Left to right: fungi E and P on oak, fungi L on sycamore



Left to right: fungi K and Q on coffee, fungi K on coffee

Note. See table 4 for descriptions of each fungi type.