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Linkind Deadwood Inhabiting Fungi to Carbon Dioxide Flux of Decomposing Coarse Woody Debris

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LINKIND DEADWOOD INHABITING FUNGI TO CARBON DIOXIDE FLUX OF
DECOMPOSING COARSE WOODY DEBRIS

by

Elyse Daub

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Ecology and Environmental Science)

The Honors College

University of Maine

August 2022

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ABSTRACT

The decomposition of wood is driven by a combination of abiotic and biotic factors, the most influential of them all are fungi. These decomposer fungi use the wood for food, digesting woody material to absorb nutrients and release carbon dioxide. Fungi are the primary drivers of decomposition in most terrestrial ecosystems; thus, the rate and degree of decomposition are greatly dependent on the community of fungi found on woody debris. However, little is known about which fungi decompose woody debris most efficiently. This study aims to investigate the decomposer fungi in the Bear Brook Watershed via molecular techniques and quantify their rates of decomposition to understand which fungi are the most effective decomposers.

ACKNOWLEDGEMENTS

I would like to thank my committee for their encouragement and invaluable support: Dr. Peter Avis, Dr. Shawn Fraver, Dr. Erin Grey, Sharon Tisher, and Dr. Ivan Fernandez. I offer my greatest gratitude to Dr. Shawn Fraver for being an unwavering source of inspiration, and his support as a mentor of mine since 2016. Without Dr. Fraver's enthusiasm and expertise in these fields, I would not have learned and grown to where I am today. Thank you to my advisor Dr. Peter Avis for supporting me through the honors thesis process and for teaching me the laboratory techniques needed for this project.

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INTRODUCTION

Coarse woody debris, fallen woody debris with a diameter greater than 10 cm, is an integral structural component for forest ecosystems by contributing to every essential ecosystem function, particularly nutrient cycling and carbon sequestration. As a tree grows, it photosynthesizes using energy from photons to transform carbon dioxide (CO₂) and water into complex sugars, and then accumulates these sugars in woody tissue as cellulose, hemicellulose, and lignin. Over decades to hundreds of years, the tree will then fall to the ground as coarse woody debris and decompose, releasing its components back into the environment. In total, the global forest carbon sink is approximately 2.4 petagrams of carbon per year (Pg C year⁻¹) globally (Pan, Birdsey, & Fang, 2011) and a net forest carbon sink of 1.1 Pg C year⁻¹ (Pan, Birdsey, & Fang, 2011) (IPCC 2022) when accounting for flux. Forests have absorbed about 30% of human emissions annually (Magnússon, 2016), therefore it is vital to understand in detail how forests lose carbon, where the carbon goes, and what forest components influence carbon loss. Coarse woody debris contains about 36- 72 Pg of carbon globally (Magnússon, 2016) and CWD increases soil carbon and nitrogen pools (Wiebe, 2015), but these estimates are highly variable and difficult to maintain accuracy of carbon storage in CWD (Russell, et al., 2015). Therefore, understanding how and where carbon is lost during decomposition of coarse woody debris is integral in forest modeling, restoration, and stability.

Decomposition of Coarse Woody Debris

Decomposition of CWD is characterized by four processes: physical fragmentation, biological transformation, heterotrophic respiration, and leaching

(Magnússon, 2016). First, physical fragmentation by bark desiccation or foraging animals facilitates detritivores and other decomposers to inhabit CWD (Magnússon, 2016). Detritivores continue to fragment CWD, further enhancing structural diversity and CWD accessibility to other decomposers, referred to as biological fragmentation. As fragmentation of the CWD increases, more surface area becomes available, and microorganisms transform complex wood compounds into soluble compounds. Respiration and biological transformation occur when CWD is consumed by microorganisms. As microorganisms respire, mass is removed from CWD and converted into CO₂ (Magnússon, 2016). The main share of the total C pool present in CWD is presumed to be respired, and the remainder is converted into biomass (Russell, et al., 2015). As fragmentation of the CWD increases, more surface area becomes available, and microorganisms transform complex wood compounds into soluble compounds (Runnel, et al., 2021) (Kubartova, Ottosson, & Stenlid, 2015). A high surface area to volume ratio combined with an excess availability of soluble compounds leads to leaching. These processes further fragment and disintegrate the CWD into pieces of organic matter in the soil (Kubartova, Ottosson, & Stenlid, 2015). Although these four steps to complete disintegration of woody material are important, the interactions of the local fungal community are the primary determinant of decay facilitated by external factors.

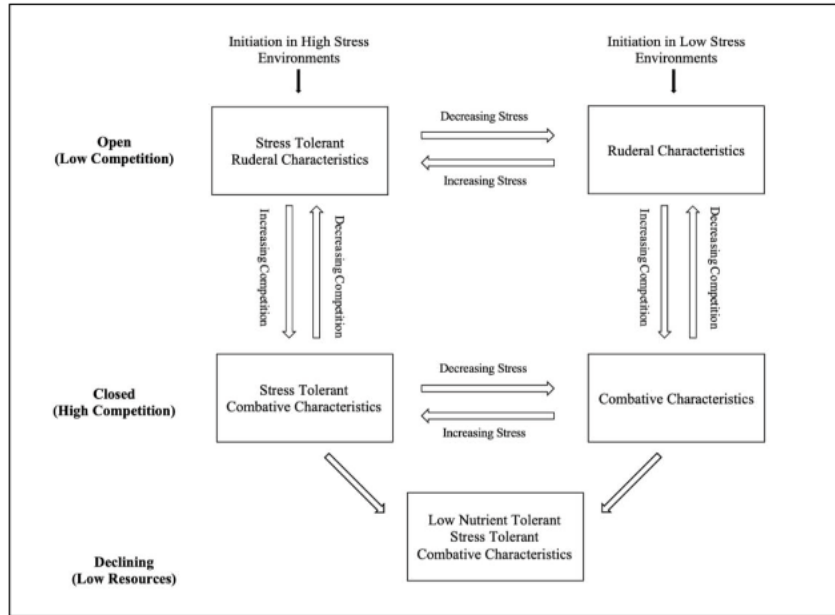


Figure 1: Diagram of the fungal community development, adapted from Boddy 2001

Community Ecology of Wood Inhabiting Fungi

The establishment and subsequent community development of coarse woody debris fungal communities is a complex multidimensional process that begins in the living, standing tree (Kubartova, Ottosson, & Stenlid, 2015) (Boddy, 2001). Trees have two primary types of tissue: the living sapwood and the nonliving heartwood. The sapwood is composed of functional tissue which contributes to a high water content and subsequent low oxygen environment. Conversely, the heartwood of mature standing trees is composed of few living cells, has a low water content and high aeration making the heartwood of a live tree more habitable to fungi than the sapwood (Boddy, 2001).

Establishment of Decay Fungi in Trees and Coarse Woody Debris. Pioneers colonize a tree first and initiate decay with mycelium or spores entering through open

wounds, roots, or as latently present fungi (Boddy, 2001). At these entry points, pioneers cause variable amounts of decay including complete/partial decomposition of the heart wood, sapwood, and canopy (Boddy, 2001) (Forrester J. M., 2011). Once the tree falls, the wood's abiotic conditions change dramatically in the sapwood whereas the heartwood remains relatively the same. The high stress conditions of sapwood alleviate when the wood dries, living cells die, and aeration improves. Subsequently, the sapwood becomes easier to colonize with fewer inhibitory chemicals than the heartwood. On or near the ground, the tree is now considered coarse woody debris and fungal establishment of the coarse woody debris initiates (Boddy, 2001).

Establishment of coarse woody debris requires the achievement of three primary goals: arrival, resource capture, and exploitation (Boddy, 2001). Fungi arrive either as pioneers or colonizers, where pioneers colonize before the tree becomes coarse woody debris and colonizers arrive on coarse woody debris. If the fungi arrive successfully, their next goal is to acquire resources through resource capture (primary or secondary). Primary resource capture is completed by both pioneers and colonizers on undecayed/uninhabited wood, whereas secondary resource capture is the acquisition of previously decayed or inhabited wood. After obtaining resources, the final phase of exploitation occurs, and fungi convert their resources into energy for maintenance or territory expansion (Boddy, 2001) (Kubartova, Ottosson, & Stenlid, 2015).

Fungi can arrive at woody debris as mycelium or propagules, where mycelium travel as networks of hyphae in the forest floor and propagules travel passively by air, water, or biotic vectors (e.g. bark beetles) (Boddy, 2001). Dispersal by propagules is

driven by chance and allows opportunities to establish across large distances, but their establishment is dependent on the combative ability of the pioneering communities. Where, if the pioneer community is dominated by ruderal characteristics with low combative ability, the propagules are more likely to establish (Boddy, 2001). Whereas encountering a pioneer community dominated by combative, stress tolerant characteristics, the propagules will have a difficult establishment when they have limited access to resources. Arrival by mycelium has limited dispersal, but the synergism between hyphae allows the translocation of resources such as water, carbon, and nutrients across areas of land, reducing the initially high energy costs of resource capture (Boddy, 2001). After arrival, fungi enter a phase of resource capture to gain territory over a volume of wood and its locked resources. Secondary resource capture by colonizers is an energy intensive process compared to primary resource capture because territory must be taken combatively. Resources within the gained territory during resource capture then undergo exploitation and are converted into energy.

Decay Fungi Community Development in Coarse Woody Debris. After establishment, decay fungi develop multidimensional communities through several pathways driven by four main forces: increasing stress, decreasing stress, disturbance, and combat (**Error! Reference source not found.**) (Boddy, 2001) (Yulien & Yucheng, 2004) (Humar, Petric, & Pohleven, 2001). The four main forces then determine community structure based on ruderal, combative, and stress tolerant characteristics. These communities can be considered open characterized by low competition, closed (high competition), or declining (nutrient deficit). Direction of community characteristic development is first dependent on the stress conditions of the substrate during community

establishment. In coarse woody debris, a high stress environment can include the harsh environment of sapwood and low stress includes following a disturbance such as windblown wood.

For wood decay fungi, forest disturbances are considered low stress environments because disturbances, such as windblown or felled wood, introduce high amounts of undecayed wood, lessening the need for stress tolerant characteristics. These low stress conditions push the community structure to favor ruderal characteristics, meaning r-selected leaning fungi that grow quickly, tolerate low nutrient conditions, and have a low combative ability. Over time competition increases, and secondary resource capture begins as the community shifts from an open to a closed community. Combative fungi then out compete the ruderal species, increasing stress and shifting the community structure towards fungi that can tolerate high stress and or those with successful combative characteristics. Eventually competition subsides, reducing combative fungi and increasing ruderal characteristics in fungi. Communities initiated during high stress conditions, such as in sapwood, tend to follow a similar but opposite pathway as those initiated under low stress. The high stress, low competitive conditions favor stress tolerant characteristics and some ruderal characteristics. If the stress remains and competition increases, competitive exclusion favors species that have stress tolerant and combative characteristics. Stress alleviation then removes stress tolerant characteristics to primarily combative species. As decay advances, acquired territory become nutrient poor and the community is comprised of low nutrient tolerate characteristics and have other ruderal characteristics.

Changes in fungal community structure are driven by metabolic rate and ecological strategy, but fungi and other organisms face a multitude of challenges within CWD that may facilitate community structure and decomposition effectiveness. Abiotic factors including volatile compounds, pH, temperature, water availability, gaseous regime, and wood size impact stress and competition between species. Additionally, microorganisms differ in their ability to degrade the primary wood components, tolerate extractives (e.g. tannins), or environmental variables such as water saturation.

Wood Decomposition Initiated in the Canopy

Variations between leaf litter and CWD decomposition may be due to in part by decomposition of pioneer communities in the sapwood of a tree's canopy (Boddy, 2001). Canopy wood is comprised of sapwood, which creates a high stress environment for wood decay fungi. Pioneers are typically S-selected (stress tolerant characteristics) and exist latently in the sapwood. If the sapwood stress alleviates (water content decreases, aeration increases), the latently present fungi begin to develop rapidly depending on the species present and on the rate, direction, and extent of stress alleviation (Boddy, 2001). Once combative secondary colonizers arrive in the canopy, their establishment depends on the wood conditions driven by the pioneers. Pioneers differ in competitive ability, so the combative ability of pioneers determine if a secondary colonizer can establish (Boddy, 2001) (Moor, Norden, Penttila, Siitonen, & Snall, 2020). But pioneers and secondary colonizers can be replaced by non-combative colonizers that are better adapted to survive adverse fluctuation conditions and then rapidly develop when conditions improve. The extent of canopy decay is variable, but complete canopy decay can occur if supported by a strong central heartwood (Boddy, 2001). More commonly, however, the

partially decayed branches fall to the ground where decomposition continues. Yet, community development after the fall is unknown, but Boddy (2001) hypothesizes that communities developed in standing wood are likely to affect patterns of colonization on the forest floor.

Biochemistry of Decay Fungi Enzymes

The major groups of decomposing fungi include Basidiomycota, Ascomycota, and some imperfect fungi. These fungi cause the three types of wood decay including white rot, brown rot and soft rot (Yulien & Yucheng, 2004). Dead wood is a complex substrate comprising a variety of components ranging from simple molecules including organic acids and sugars to complex biopolymers including cellulose, hemicellulose, and lignin (Floudas, et al., 2012) (Brethauer, Shahab, & Studer, 2020) (Carpita & McCann, 2020).

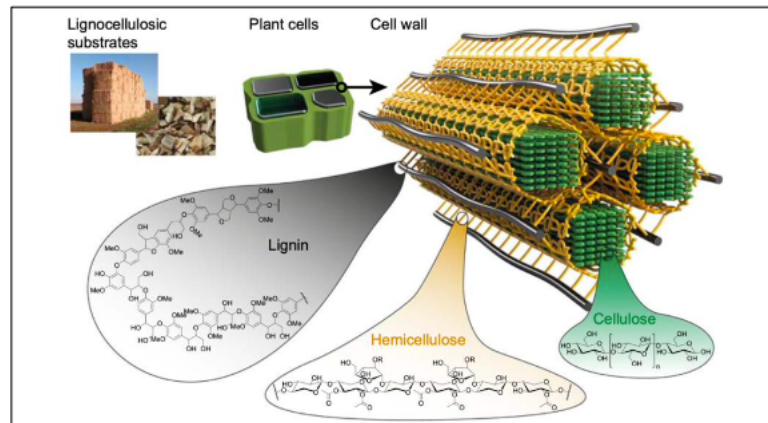


Figure 2: Structure of Lignocellulosic Plant Cell Walls (Brethauer, Shahab, & Studer, 2020)

These complex biopolymers are the factors that make wood highly resistant to decay, especially lignin. Lignin is a heterogeneous polymer that provides structural support, strength and rigidity to wood while surrounding cellulose and hemicellulose to

protect them from microbial attacks (Floudas, et al., 2012) (Cornwell, 2009). To access cellulose, lignin must be degraded first by enzymes such as peroxidases. Peroxidases (also referred to as peroxide reductases or ligninolytic peroxidases), is a wide group of catalytic enzymes which increase the rate of oxidoreduction reactions (Albuquerque, Soussa Silva, & Macedo, 2019). Peroxidases can be produced by bacteria and fungi, however those produced by fungi (fungal class II peroxidases) are generally regarded as more efficient in breaking down lignin (Albuquerque, Soussa Silva, & Macedo, 2019). Fungal class II peroxidases can be categorized into three subgroups including lignin peroxidases, manganese peroxidases and versatile peroxidases. Lignin peroxidases oxidize the major non-phenolic structures of lignin. Manganese peroxidases oxidize manganese (Mn^{2+}) into highly reactive manganese

(Mn^{3+}) that reacts with lignin creating unstable free radicals and eventually disintegrate spontaneously. The third type of peroxidases, versatile peroxidases, are capable of the same catalytic abilities of both manganese and lignin peroxidases (can oxidize Mn^{2+} and non-phenolic compounds) but utilize different mechanisms and capabilities (Abdel-Hamid, Solbiati, & Cann, 2013) (Albuquerque, Soussa Silva, & Macedo, 2019). White rot species are capable substantial lignin decay by synthesizing peroxidases, whereas brown rot species lack these enzymes and only modify lignin not sufficiently degrade it. Typically, species with less aggressive decay enzymes depend on the prior activity of aggressive decayers to generate more degradable compounds, create suitable abiotic conditions, or to detoxify substances that limit their activity. (Harmon, et al., 1986)

The second complex biopolymer that must be broken down is cellulose. Similarly, to lignin, specialized enzymes are needed to target cellulose, however cellulose is less structurally diverse among plants as compared to lignin. The catalytic enzymes that target cellulose include oxidoreductases, carbohydrate active enzymes, and glycoside hydrolases (Floudas, et al., 2012). Carbohydrate active enzymes act on crystalline cellulose and are abundant in white rot fungi and less abundant in brown rot fungi and ectomycorrhizal fungi (Floudas, et al., 2012). Glycoside hydrolases, present in white rot and relatively absent in brown rot, include cellobiohydrolases that are involved in the breakdown of crystalline cellulose and cellulose binding modules. Simultaneously breaking down cellulose and removing cellulose binding molecules increases the concentration of the enzymes on the surface of cellulose (Floudas, et al., 2012), further accelerating the rate of cellulose decay.

As wood further undergoes delignification and cellulose degradation, the energy and resources they obtain are transformed into biomass and later leached from the CWD. Leached residues from CWD are enriched in complex, microbial derived compounds such as chitin, beta-glucan, melanin, glomalin, fungal lipids, bacterans, glucosamine, galactosamine and mumaric acid (Magnússon, 2016) (Schulze, 2000). Fungi construct these compounds by diverting carbon resources from CWD into stabilized biomass, rather than releasing excess carbon through respiration as carbon dioxide. Therefore, CWD may further enhance and stabilize forest carbon pools (Magnússon, 2016) (Schulze, 2000). However, stabilizing carbon in this fashion is determined by the local fungal community and their competition/stress regime. For example, a community that diverts more converted energy into biomass would likely be under low competitive

pressures and low amounts of stress to minimize energy diverted towards combative strategies.

Abiotic Determinants of Communities

As a kingdom, the distribution and abundance of fungi is commonly dictated by two factors: moisture and temperature (Runnel, et al., 2021) (Purahong, et al., 2018). However, the source, amount and degree of variability vary among groups and species to species, and wood decay fungi are no exception. This paper will focus distribution and abundance to three primary scales: local (log), landscape (forest the log resides in, such as The Bear Brook Watershed), and regional (Northern New England).

Determinants at the Local Scale. At the local scale the biotic and abiotic factors that control distribution and abundance of fungi are moisture content, stage of log decomposition (substrate condition), and competition. Few studies have examined the relationship between fungal communities and local scale factors with varying results, often with the same uncertain conclusions that it remains difficult to tangle out if the fungi are creating optimal substrate conditions or if the substrate conditions are determining the fungal communities. These factors are further complicated by other organisms, since deadwood is considered a hot spot for biodiversity of millions of species including bacteria, fungi, plants, and animals (Stokland, Siitonen, & Jonsson, 2012) (Purahong, et al., 2018) (Schulze, 2000) (Yulien & Yucheng, 2004). Common substrate conditions that have been explored include wood moisture content, level of wood permeability, wood pH, tree species, and wood volume (local and regional). Studies have shown these factors to influence community composition but lack direct consistency

between communities. pH and tree species on the local scale have been shown to have the greatest influence, however other studies have had differing results (Purahong, et al., 2018) (Humar, Petric, & Pohleven, 2001) (Kok, Haverkamp, & Van Der Aa, 1992). In Purahong et al (2018) wood pH was the only wood physiochemical property they assessed that consistently corresponded to community composition, including between conifer and deciduous species. The only physiochemical property that consistently correlated to the communities in deciduous and conifer species was wood pH. In deciduous logs, the tree species and physiochemical wood properties were important factors, but in conifer logs pH was the only physiochemical property to consistently correlate to communities. Kok, Haverkamp, and Van Der Aa (1992) identified that pH is a factor in determining mycelial growth, decomposition ability of plant materials and enzyme production by fungi, however, this study focused on fungi growing on aquatic plants (non woody) and explains that pH could a contributing factor, and not that it has a deterministic impact. A difficulty in measuring pH is that narrowing down the source of pH fluctuation may pose difficult. As previously explained, as fungi and other organisms break down wood, other resources are released into the system and could impact pH based on the organisms producing them. For example, in Humar, Ptric and Phleven (2001), their aimed to see how wood pH changes in result to different wood rotting fungi. The study used three different species of brown rot fungi and found that the brown rot fungus *A. vaillantii* decreased the pH of wood before mass loss occurred, and the white rot fungi *R. versicolor* and *S. commune* caused a slight increase of wood pH. The results suggest that a decrease of wood pH may be an early indicator of decay by brown rot fungi. The discrepancies in these studies may be due to wood decomposition stages. The

two main factors that contribute to community initiation are stress and competition. Therefore, a study such as Purahong et al (2018) that focused on logs in the first three years of wood decomposition, could be skewed to more readily find the initial stress that determines the community and identify that as a factor that determines community structure and composition. For instance, if there were no dramatic stresses on a community, less dramatic local conditions could prevail as a stressor and then shape the long-term community. Alternatively, if a more intense stress is imposed on the community than unideal pH, then pH would not be a determining factor. It is important to note, however, that Purahong et al (2018) extensively investigated numerous wood physiochemical properties across both conifers and broadleaf species and is one of the most recent studies to do so. Therefore, their results are more representative of factors across landscape scales. Regardless, to untangle these discrepancies more studies should include more detailed analysis of pH to determine if it is a factor that determines communities or a community indicator.

Determinants at the Landscape Scale. At the landscape and regional scale, species distribution and abundance are controlled by woody debris volume, total forest cover and forest connectivity (lack of fragmentation) (Purahong, et al., 2018) (Runnel, et al., 2021). Runnel et al (2021), assessed factors and scales which shape fungal communities on spruce logs via metabarcoding. Suggesting that substrate amount (log volume) and forest connectivity support fungal diversity in dead wood. Runnel et al (2021) additionally found more fungi in older stands (>80 years old), however stand age is subjective and often misrepresents the forested ecosystem. Woody debris volume on landscape scales alters the stress and competition regimes for fungi, and total forest cover and connectivity

also impact these regimes. Woody debris, forest cover and connectivity particularly impact the metapopulation dynamics of fungal communities. Metapopulation theory is concerned with patchiness of populations in an area and how the separation and spacing of patches impacts populations, where logs may behave similarly to patches and act as dispersal sources. In addition, there are common colonization and extinction event dynamics that are correlated with species degree of specialization (Moor, Norden, Penttila, Siitonen, & Snall, 2020). Where the more specialized a species was the more sensitive their colonization rates were to habitat conditions (log volume and stand age), and not generalist species (Moor, Norden, Penttila, Siitonen, & Snall, 2020). Therefore, log volume could determine some variations of community structure and the ratio of specialist/generalist species may indicate habitat conditions. However, the habitat conditions may not be deterministic of the fungal communities, and this is a difficult factor particularly in terms of future carbon pool modeling (Bradford, et al., 2014).

Measuring Decomposition of Coarse Woody Debris

CWD constantly undergo physical and chemical changes, but decay is not linear and decomposition rates change during different stages of decay (initial, intermediate, and advanced) (Kubartova, Ottosson, & Stenlid, 2015) (Runnel, et al., 2021), community stage, abiotic factors, and or a combination of these. But, determining how these factors relate and interact with each other, particularly in the progression of decay, remains uncertain in the literature. Except a common consensus that wood decomposition is largely determined by various fungi, the rate, extent, and pattern of decay depends on community composition, and the ability of individual decomposers under microenvironmental conditions.

CWD decomposition has been measured in many ways, but most studies use methodologies including chronosequences, fitting decay models, and direct measurement of respiration fluxes (Russell, et al., 2015). Chronosequences with the fitting of decay models is beneficial because it eliminates the problem of studying these systems over long periods of time (Russell, et al., 2015). However, these studies use changes of CWD characteristics over time as estimates of decomposition, which do not account for losses caused by respiration, leaching, and uptake into microbial biomass. Alternatively, respiration can be directly measured by installing respiration chambers or collars on logs to quantify respiratory CO₂ fluxes. Current research of CWD respiration rates have indicated that the C stored in CWD contributes significantly to the forest C cycle.

Thesis Research Objectives The Bear Brook Watershed in Maine

The Bear Brook Watershed in Maine is home to a two paired, forested stream watershed system on the southeastern slope of Lead Mountain in Beddington, Maine. Beginning in the 1980's, research at Bear Brook proliferated as with multiple collaborative studies by the University of Maine, USDA Forest Service, the U.S. Geological Survey, and other institutions. Despite its unremarkable location in Maine, Bear Brook's whole ecosystem experimental manipulations to study the impacts of acid rain makes it a unique study area. Beginning in 1989, additions of nitrogen and sulfur as diammonium sulfate were applied to the West Bear watershed bimonthly via helicopter from November 1989 to August 2016 and the East Bear watershed remained untreated. Research at Bear Brook encompasses a wide range of topics from carbon sequestration to base cation depletion across the paired watersheds, and a vessel to study this through is in coarse woody debris (CWD).

In Gough et al. (2007) sequestration respiration rate from CWD was 10 times lower per unit mass than from leaves despite their relatively equal amounts of C mass. Additionally, previous research at the Bear Brook Watershed in Maine has shown that nitrogen addition slowed the decay rate of leaf litter, however, the effect of nitrogen addition on wood decay rates remained unknown. To measure nitrogen's impact on the decay rate of coarse woody debris, decay stakes that act as a proxy for wood decomposition were installed (Figure 4) at sugar maple logs on Bear Brook in July 2016. The proportion of mass loss can then be measured by the decay stakes calculating the difference between their initial and final mass. However, the decay stakes may provide insight into the fungal community of a decomposing log, and if the decomposing log influences the fungal community on nearby decomposing woody debris.

Previous research has identified gaps in the knowledge of decomposition of coarse woody debris, and this study aims to add perspective and answer the following questions: What is the local fungal community composition on a decomposing log? Is there a fungal community change moving away from a log? What is the relationship between the fungal communities surrounding the log and the log itself? Is there a relationship between the log's fungal community composition and carbon dioxide flux?

To accomplish this, four sets of data have been collected: (1) the DNA of fungi on sets of experimental decomposing logs for species identification, (2) the DNA of fungi for identifying the variety of species on proxy logs called "decay stakes" (deployed across a gradient from the experimental log), (3) the mass loss of each decay stake as an

indicator of overall log decomposition, and (4) the carbon dioxide flux from each experimental log.

MATERIALS AND METHODS

Field Experimental Design: Log Locations and Decay Stake Layout

In July 2016, sugar maple decay stakes (approximately 1 in x 1 in x 7 in) were installed nearby decomposing sugar maple logs. The logs in this study were any sugar maple logs within Bear Brook Watershed with a decay class of 3. Data collected of the logs included the GPS location, watershed type, decay class, log length, DBH, slope, and angle. Four 1 m decay stake transects were placed perpendicular to the log on top of the forest floor and securely pinned into the soil to ensure the stake stayed in place (Figure 4). After 5 years, the stakes were removed in July 2022.

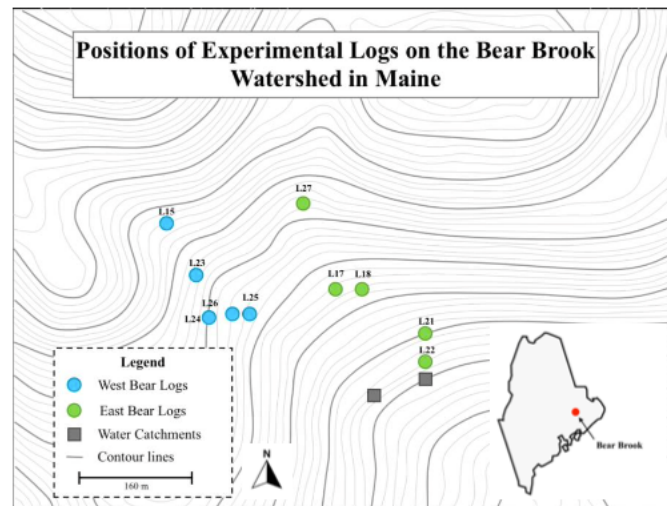


Figure 3: Bear Brook resides on Lead Mountain in Beddington, Maine. The logs for this study reside in both West Bear (blue) and East Bear (green). Each watershed has an associated water catchment site that have been monitored since 1987, before chemical treatments of West Bear.

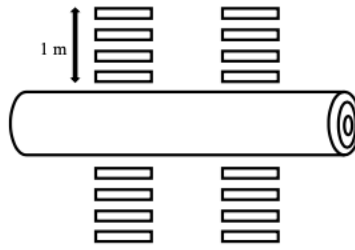


Figure 4: Digital illustration of the four transect decay stake layout. Each set of four decay stakes were placed one meter out from the log, equidistantly.

Fungal Species Identification

Experimental Log Woodchip Sampling

After carefully removing the bark using a sterilized chisel, the wood tissue was removed by drilling into the log at approximately pith height parallel to the ground. Woody tissue that remained on the drill bit was placed inside a plastic bag. In cases where a log was wet or otherwise difficult to drill an alternative removal method was applied. After the bark was removed, sterilized tweezers were used to remove woody tissue and placed into a plastic bag. Plastic bags containing these samples were then placed into a -20 oC freezer.

Decay Stake Woodchip Sampling

Following the decay stake removal, half of the decay stakes were sampled for wood chips. After their removal, stakes intended for molecular work were placed in individual plastic bags on ice overnight. Every stake was first lightly cleaned of any heavy debris and a thin outside layer of the stake was removed with a scalpel to expose the inner wood. Next, a drill with a sterilized 1/8-inch drill bit was drilled into the stake

to remove woody tissue. The woody tissue that remained on the bit was then placed into a 4 mL centrifuge tube and frozen at 20°C. In cases where the stakes were too wet, crumbly, or other ways difficult to sample with the drill then tweezers were used to remove the woody tissue in place of the drill. After sampling, any woody tissue removed from the stake not set aside for later DNA testing was placed back with the stake. Any sampling which removed volumetrically greater woody tissue than would be lost with three drill hole was noted. Once sampled, each stake was oven dried and weighed.

DNA Extractions

All woodchip samples (both from the decay stakes and the logs) were pulverized into a flour-like texture using a mortar and pestle after the sample was covered in liquid nitrogen. After pulverization, each sample underwent DNA extraction using the Thermo Scientific GeneJET DNA extraction kit and following the kit's protocol.

Polymerase Chain Reaction

To initialize the polymerase chain reaction (PCR), a Master Mix was made in a UV sterilized hood, combined with the DNA extracts, and then transferred into a thermocycler. The PCR UV hood was first sterilized with RNAway and UV light for 15 minutes. The total amount of Master Mix ingredients was calculated by multiplying the ingredient volume per reaction (μL) with the number of total reactions (sum of the number of samples and positive and negative controls). Once the ingredients thawed on ice and were vortexed, the Master Mix was made and distributed into PCR tubes. The template DNA was then added to the PCR tubes with Master Mix and placed into a

thermocycler. Following Morvan et al (2020), the thermocycler program ran at 95 oC for 3 minutes, 35 cycles of amplification (denaturation at 94 oC for 30 seconds, annealing at 53 oC for 45 seconds, and extension at 72 oC for 1 minute, and a final elongation at 72oC for 10 minutes.

Ingredient	Volume per Reaction (µL)
PCR H ₂ O	4
10 µM KYO2	1.25
10 µM KYO3	1.25
Bio-Rad iTaq Super Mix	12.5
BSA (Bovine serum albumin)	1
Template DNA	5

Table 1: Master Mix recipe for PCR

Gel Electrophoresis

The electrophoresis gel was a 1% agarose gel solution with 0.5g of Agarose and 50 mL of 1X TAE EDTA. The primary ingredients were first measured out into a beaker and microwaved to fully combine. Once the solution was clear, the beaker rested until warm to the touch. Then 10 µL of SyberSafe was added, and the solution was slowly poured into a gel box and left to set until solidified. 1X TAE EDTA was then poured into the gel box until covering the fully set gel. The 100bp ladder along with the PCR product and negative control combined with Blue/Orange 6x loading dye were transferred into the empty gel wells. The gel was removed when the blue lines traveled approximately halfway across the gel then photographed over UV light.

Sequencing and Species Identification

The DNA extracts were sent to a sequencing lab, which then returned a series of amplified sequence variances (ASVs). The ASV data was then uploaded to GenBank Nucleotide BLAST which returned species identifications. Fungi identifications were then determined based on the returned BLAST data.

Carbon Dioxide Flux Measurements

Installation of CO₂ Flux Collars

CO₂ collars were installed on 8 of the 10 experimental logs, with two logs having three collars and the remaining six with one CO₂ collar. These collars were manually installed using a custom shaped PVC pipe and silicon adhesive. Collars were installed in line with two decay stake transects on bark covered wood laying on the forest floor. Before aligning the adhesive, the exposed bark was cleaned using a brush to maximize contact with the bark. Once the bark was prepped for installment, silicon was dispensed onto the base of the PVC pipe and carefully placed onto the marked section of bark. Additional silicon adhesive was added around the bottom outside edge to ensure secure attachment to the wood and left to rest for at least 24 hours. In addition to the collars, iButtons, temperature recording devices, were installed, and secured under a heat reflective shield on the bark next to the collar on its north side.

CO₂ Flux Measurements

Carbon dioxide flux was measured from the installed collars three times in July and August 2021. At each collar, a custom fit lid connected with airtight tubing to a LiCOR CO₂ analyzer was placed over the collar and secured on its sides using one

bungee cord. Once the lid was placed and secured, the LiCOR connected to the data recording app FluxPuppy was initiated and collected data for three minutes. If leaks were present in the system, silicon adhesive was applied to gaps or holes from the original installment. In the case where a collar was removed by curious bears, crows or from bark sloughing off, the collars were reapplied to the log. During the three-minute recording window, the bark temperature at the iButton and soil moisture were recorded.

RESULTS

These results show variability across multiple values. When comparing carbon dioxide flux to mass loss across all logs, there is an apparent disparity because the East Bear and West Bear groups. Where, the East Bear samples have greater variance than the West Bear samples do, comparatively (Figure 5). These results also indicate no positive trend with mass loss and carbon dioxide flux as expected. Additionally, comparing the proportion of Ascomycota species with Basidiomycota species across watersheds shows that Basidiomycota species hold approximately 0.77 of the total ASV (amplified sequence variance) abundance values, with the remaining portions held by Ascomycota and Alveolates (protist group). Interestingly, although results between the two watersheds do not differ greatly among Basidiomycota, the West Bear ASV abundances are more split between the Ascomycota and Alveolates groups (Table 2).

Across all logs, the number of sequences were primarily composed of Ascomycota followed by Basidiomycota and Alveolates (Figure 6). However, when comparing the percentage of ASV abundances the results are flipped, with Basidiomycota dominating followed by Ascomycota and Alveolates (Figure 7). With exception for L22 and L26, which are similar in taxonomic share between the number of sequences (Figure 6) and ASV abundance (ASV 7). When comparing the log ASV values and abundances to those across the decay stakes, preliminary results show that groups of stakes do partially cluster together. Stakes and logs may also share similar taxonomic grouping, where L22 ASV abundances are unusually high in Ascomycota the corresponding stakes are high in Ascomycota as well.

When comparing these results to CO₂ flux, preliminary results do not indicate a correlation between the major fungal groups. However, there was a correlation between species richness, CO₂ flux and mass loss. Such that when species richness is counted for samples with an ASV > 100, there is a 0.4216 correlation with mass loss and a -0.2622 correlation with CO₂ flux. Additionally, when excluding ASV values to >1000, these correlations increase substantially to 0.6152 and 0.8589, respectively.

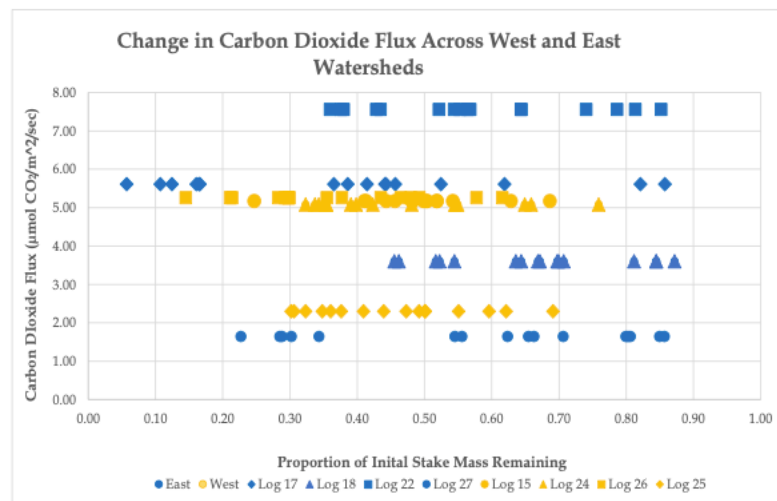


Figure 5. Carbon Dioxide flux across log mass loss.

Phylum	Total ASV Abundance (%)	East Bear ASV %	West Bear ASV (%)
Ascomycota	0.19	0.2074	0.1612
Basidiomycota	0.78	0.7760	0.7777
Alveolates	0.04	0.0166	0.0611

Table 2. ASV Abundances across taxonomic groups in each watershed.

	Mass Loss Correlation	CO ₂ Flux Correlation
Sp. Richness (ASV>100)	0.4216	-0.2262
Sp. Richness (ASV>1000)	0.6152	0.8589

Table 3. Correlations of species richness of varying minimum ASV values (ASV > 100 or ASV >1000) for mass loss and CO₂ flux.

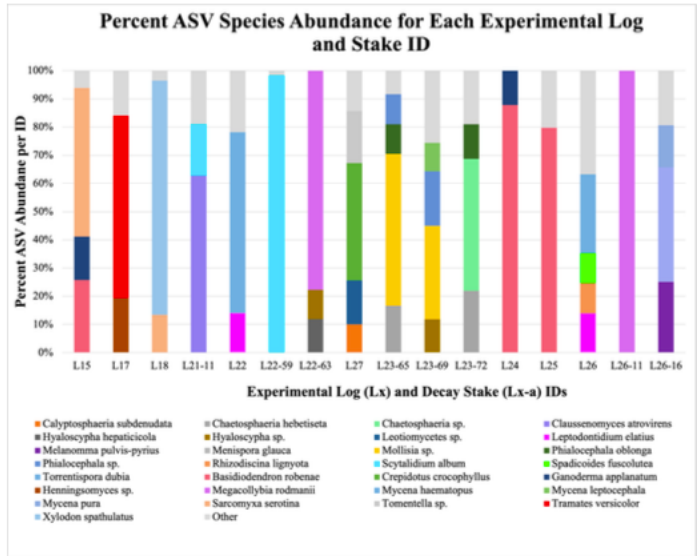


Figure 6. The total proportion by per ASV of species abundances for each experimental log and stake in this study.

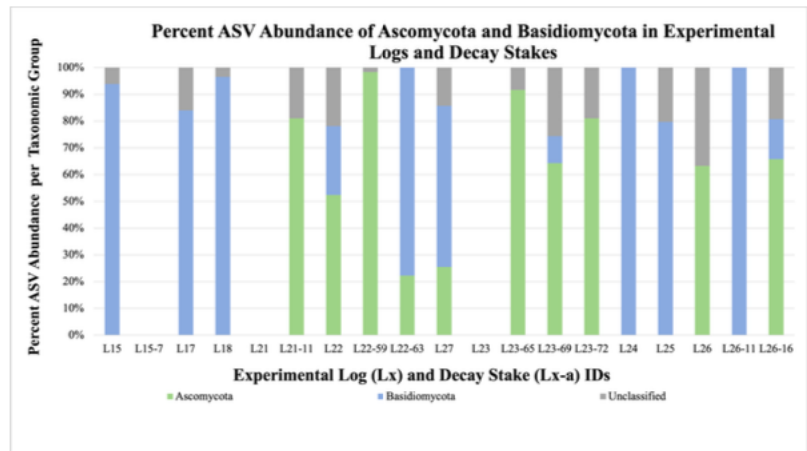


Figure 7. The proportional abundances of Ascomycota and Basidiomycota in the experimental log and decay stakes. Unclassified samples were samples which either did not fall into a category of fell under less than 10% of the total ASV abundance.

ID	Phylum	Ecology
<i>Rhizodiscina lignyota</i>		
<i>Leptodontidium elatius</i>		
<i>Leotiomycetes sp.</i>		
<i>Torrentispora dubia</i>		
<i>Basiodendron robenae</i>		
<i>Xylodon spathulatus</i>		
<i>Ganoderma applanatum</i>		
<i>Trametes versicolor</i>		
<i>Tomentella sp.</i>		
<i>Crepidotus crocophyllus</i>		
<i>Mycena haematopus</i>		
<i>Sarcomyxa serotina</i>		
<i>Henningsomyces sp.</i>		
<i>Sporadotrichida sp.</i>		
Other (Value <10% Total Abundance)		



Table 4. Top 10% of species unique to experimental logs with their classification and documented ecological roles.

	Mass Loss	CO2 Flux	Gap Fraction 90	Wood Temperature	Log Soil Moisture Content
Species Richness	-0.2293	-0.3476	-0.1404	-0.6891	0.8474
ASV Abundance	-0.5361	-0.1188	-0.2233	0.1278	0.1558
Mass Loss		0.0185	0.1746	-0.1151	-0.2142
CO2 Flux			-0.2221	0.1492	-0.4192
% Ascomycota			-0.1766	-0.7722	0.4458
% Basidiomycota			0.1766	0.7722	-0.4458
Gap Fraction 90				-0.1388	-0.3002
Wood Temperature					-0.6080

Table 5. Correlations between experimental log factors.

	% Ascomycota	% Basidiomycota	ASV Abundance
Species Richness	0.3333	-0.3333	0.5044
ASV Abundance	0.0408	-0.0408	
Mass Loss	0.0620	-0.0620	
CO2 Flux	0.2221	-0.2221	

Table 6. Correlations between experimental log and decay stake factors.

CONCLUSION

Coarse woody debris is an underestimated component of forest ecosystems. However, without the power of fungi and their uniquely complex enzymes the landscapes we know today would not exist. Fungi establish on trees before they become coarse woody debris, however the system changes in response to stress (presence or absence), competition and disturbance. These changes in fungal community structures are driven by their metabolic rates and ecological strategies, but abiotic and biotic challenges within woody debris also facilitates community structure. This complex study system was examined at the Bear Brook Watershed in Maine, using decay stakes and experimental logs across two watersheds. The results of this study partially supports conclusions from the literature, however further analysis of all decay stakes are needed to assert strong conclusions. Despite this, preliminary results are promising and indicative of further investigation.

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AUTHOR'S BIOGRAPHY

Elyse Daub is a budding plant parent, who first remembers loving science at five years old. Affectionately nick named “The Frog Hunter,” Elyse started her science career catching frogs in her frog rain boots at her lakeside home in Maine to answer the question: How many frogs in the lake are green? Elyse began formally working towards her goal of becoming a scientist in 2014 when she joined the Bangor High School STEM program. During this time at Bangor High, she was awarded a full tuition scholarship to the University of Maine as a Maine Top Scholar in 2017.

Although her life has been marked with success, Elyse’s life is based in tragedy. Born with a rare congenital vascular malformation in 1999 and surviving her father’s suicide in 2004, Elyse has endured and persevered to show anyone, and herself, that through loss life finds a way. In her personal life, Elyse is on a new path of self-acceptance following an ADHD diagnosis and battling depression and anxiety. Elyse currently finds her career aspirations in the intersections between science and government. She aims to become a leader and advocate for scientific literacy by focusing on science education expansion and raising awareness of learning disabilities across all industries.