

# Global Distribution of Wood-Decay Fungi: Patterns Without Predictability

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## Abstract

There are three principal types of wood-degrading fungi: white-rot, brown-rot, and soft-rot. A basic role of wood-degrading fungi in forest ecosystems is to recycle the carbon stored by autotrophic organisms. Differences in the pathway through which each type of fungi degrades wood determines whether the carbon is released to the atmosphere as CO<sub>2</sub>, a greenhouse gas, or is recycled to the soil. Understanding the distribution and dominance of the different wood-decay fungi is pertinent to predicting their effect on the carbon cycle and a prerequisite for any type of mitigation strategy. Early theories attributed distribution to latitudinal geography based on correlative analysis. Attention then turned to the effects of climate, specifically moisture content and temperature, on this distribution. The prevailing theory is the connection between the wood substrate – either hardwood or softwood – and the fungal rot type, though recent evidence has called the absoluteness of this relationship into question. Current research is turning its focus to chemical growth factors of fungi such as nitrogen and phosphorous to see if these factors can explain rot type distribution. At present, many patterns between variables and distribution have been identified, but consistent counterexamples limit their predictability.

## Introduction

The basic role of wood-degrading fungi in the global ecosystem is to recycle the carbon removed from the atmosphere via photosynthesis by autotrophic organisms (Gilbertson, 1980). While many economically based views of wood-degrading fungi are reasonably negative, their value to natural ecosystems and the role they play in the global carbon cycle is substantial. There are three principal types of fungal decomposers in rotting wood: white-rot, brown-rot and soft-rot (Eriksson et. al, 1990). Some refer to these three as “true wood-degrading fungi” because of their unique ability to overcome the lignin barrier in some manner and cause decay of the wood cell walls (Daniel, 2016). These fungi often co-exist in nature, which creates a balance in carbon turnover because of their various abilities to either recycle or release carbon to the atmosphere (Schilling et. al, 2020). However, if one type of fungi were to begin to dominate, and their global distribution change due to a corresponding variation in some related factor, there could theoretically be a tip in the carbon balance maintained by these fungi. This would potentially lead to dramatic impacts upon the carbon cycle and climate. Thus, understanding the distribution of these fungal decay types is pertinent to maintaining this balance.

As the dominant forest decomposer, fungi release the Earth’s largest pool of biotic carbon – wood (Schilling et. al, 2020). Via different decay methods, each fungal decay type alters wood solubility, strength, and the wood lignin in unique ways that leads the carbon in the wood towards dramatically different fates that may alter CO<sub>2</sub> emission in a given forest (Brischke et al., 2008). White-rot fungi are characterized by their wood degrading process that decomposes the lignin present in wood so the fungus can access the energy-rich, but embedded, cellulose and hemicellulose. Brown-rot fungi meanwhile have developed an alternative path to the desired cellulose and hemicelluloses that leaves the lignin relatively intact. Soft-rot fungi are less

researched and as a result less understood but are characterized by a wide diversity of species that generally attack through a process known as cavitation.

Because of the differences in their degradative pathways, monitoring the proportion of white to brown-rot fungi present is critical to understanding the fate carbon released from wood in forest ecosystems. Carbon dioxide is a greenhouse gas, and its emission from wood lignin (20-25% of wood, by weight) will increase dramatically if a brown-rot-dominated system transitioned to white-rot dominance. Of critical importance to the carbon cycle, white-rot fungi decay lignin and carbohydrates present in both softwoods and hardwoods to CO<sub>2</sub> and H<sub>2</sub>O (Daniel, 2016). Meanwhile, CO<sub>2</sub> release by brown-rot fungi is generally small, with many brown-rot fungi having been found to release less than 2% of the carbon present in the substrate (Eriksson et. al, 1990). At stake is roughly 80% of the world's 575 petagrams of biotic carbon, or 450 petagrams ( $450 * 10^{15}$  grams).

## White-Rot Fungi

There are over 10,000 documented species of white-rot fungi, all of which possess a varying ability to degrade lignin, hemicellulose, and cellulose (Kang et al., 2007). They are the most abundant degraders of wood in nature and their general route to accessing the hemicellulose and cellulose embedded in the lignin matrix is driven by their unique ability to remove the lignin (Hammel, 1997). In North America, white-rot fungi account for 94% of the 1600-1700 species of wood-rotting fungi (Blanchette, 1991). Variation among the 10,000 species however is substantial, and the proportion or order of decomposition of the lignin, cellulose, and hemicellulose is not uniform (Eriksson et. al, 1990). Research and understanding of white-rot decay is dramatically more advanced than all other forms of wood decay, driven by the

possibility to harness the lignin-degrading enzymes used in white-rot decay for applications in biotechnology and the pulp and paper industry (Daniel, 2016).

White-rot fungi are also the oldest of the wood-degrading fungi, with fossil records indicating that white-rot occurred as early as the Triassic period (Blanchette, 1991). Most white-rot fungi are a part of the taxonomic group known as the basidiomycetes – or colloquially as the higher fungi – and produce decay in hardwoods and softwoods across the globe (Daniel, 2016). Some white-rot fungi belong to the ascomycetes and can produce decay in hardwoods, though they are unable to produce the same level of decay and are unable to attack softwoods (Gilbertson, 1980). This diversity in white-rot fungi is seen on both a macroscopic and microscopic scale (Eriksson et. al, 1990). The most distinctive difference among the white-rot fungi is whether they remove all cell wall components simultaneously, proportional to their existence in the cell wall, or if they selectively degraded lignin (Blanchette, 1991). This has led to the further distinction of classifying fungi that cause white-rot decay as either simultaneous rot or selective rot fungi. Further, it is possible that the order in which the lignin, cellulose and hemicellulose are degraded not only varies between white-rot species but also between what type of wood substrate the fungi is attacking (Eriksson et. al, 1990). The most visual distinction of white-rot fungi is the bleached white color that occurs during the stages of advanced decay, hence where it receives its name (Eriksson et. al, 1990). Visually, the wood takes on a white-stringy appearance that with pockets of white. In general, selective degradation of lignin leads to the most visible white color while simultaneous rot leads to a more tan appearance. When a fungus exhibits both forms, a white-tan mottled appearance has been seen.

## Morphological & Chemical Changes

It is widely supported that white-rot fungi colonize wood quickly and first establish in the cells of the wood xylem, with the ray parenchyma cells the first to be colonized (Eriksson et. al, 1990). White-rot fungi then move from cell to cell via hyphal penetration of pit structures or directly through cell walls with little difficulty (Eriksson et. al, 1990). Simultaneous rot by white-rot fungi is most commonly observed in softwoods, where cell wall degradation begins with the cell lumen and proceeds through the secondary cell wall, degrading the entirety of the S3, S2, and S1 layers in succession all the way through to the middle lamella (Daniel, 2016). This leads to holes in the wood developing where the entire cell wall was destroyed, referred to as cavities. This form of sequential attack of the secondary layers is also seen in white-rot fungi attack of hardwoods by ascomycetes (Liese, 1970). However, while complete removal of the S3 and S2 layer are observed, the S1 layer and compound middle lamella are only sometimes eroded. This leads to a more non-uniform erosion throughout the wood and may lead to instances where some fibers are substantially eroded while others are entirely intact. In advanced decay stages, the wood degradation becomes more uniform and more closely resembles simultaneous white-rot attack on softwoods, though variability is still seen. During simultaneous rot, cellulose, lignin, hemicelluloses, and extractives are attacked (Daniel, 2016).

On the other hand, white-rot fungi that preferentially degrade lignin cause substantially different morphological changes to the cell wall (Eriksson et. al, 1990). In this form of decay, the lignin is selectively removed from the entirety of the cell wall, which leads to an extensive loss of the middle lamella, and the separation of cells from one another (Daniel, 2016). Because lignin is predominantly in the middle lamella and not in the secondary wall, the majority of the secondary wall remains. In softwoods, the entirety of the middle lamella is often removed but

cell wall integrity is maintained (Eriksson et. al, 1990). In hardwoods, the middle lamella is typically not entirely degraded, though delignification does lead to separation of fibers and vessels. In both cases, the crystalline structure of cellulose is not destroyed, which is a direct contrast from white-rot fungi that cause simultaneous rot (Eriksson et. al, 1990). However, lignin cannot be the sole source of energy for white-rot fungi (Hammel, 1997). Rather, selective delignification of wood appears closely associated with a reduction in the hemicellulose content of the wood (Daniel, 2016). This correlation is assumed to be related to the formation of lignin-carbohydrate complexes, which explains how hemicellulose, which is then used as the energy source for the fungi, can be degraded while the cellulose remains almost fully intact (Eriksson et. al, 1990). Not until after all the lignin is removed is cellulase activity observed, which removes cellulose from the secondary layer.

Though selective delignification leads to morphological and chemical characteristics of wood that are dramatically different from simultaneous attack, both forms are considered white-rot degradation because of several recorded instances where a single fungus produces both types of attack on the same substrate (Daniel, 2016). Moreover, the quantity of simultaneously decayed wood and delignified wood varies substantially among strains of even the same fungus, and particularly between different fungal species and substrates (Eriksson et. al, 1990 – Blanchette 1984). Most theories attempting to explain the difference revolve around environmental factors and the nitrogen concentration in wood, which will be discussed later (Dill & Kraepelin, 1986).

Because of white-rot fungi's unique ability to decay lignin, it follows that measuring lignin loss relative to some other attribute would allow for simple classification of a fungi as a white-rot. At present, this method is known as lignin loss to relative to density loss, or the L/D ratio, which provides an index of carbohydrate selectivity vs lignin selectivity (Schilling et al.,

2015). White-rot fungi then correspond to a high L/D ratio – specifically above 0.8, while brown and soft-rot fungi have a lower L/D ratio. White-rot species have been shown to remove lignin at substantially quicker rates than brown-rot – roughly 12 times faster (Worrall et. al., 1997).

Further, selective rot white-rot fungi degraded lignin nearly twice as fast as white-rot fungi that cause simultaneous rot (Schilling et. al, 2020). It has been found that of the white-rot fungi, those that cause the most serious decay selectively delignify wood (Blanchette, 1991).

### Wood-Degrading Enzyme System

While visibly white and brown-rot decay differ in the visual appearance of degraded wood, at the molecular level however, the difference between fungi that cause white and brown-rot decay is far more complex. The key difference between the two is the usage of different enzyme systems that are geared towards their preferential form of attack. While the white-rot fungi are clearly very heterogenous, they do have a common capacity to produce extracellular enzymes capable of oxidizing phenolic compounds related to lignin – their defining, and remarkably unique, characteristic (Daniel, 2016). While the order of preferential attack does vary, research has found that wood polysaccharides – cellulose and hemicellulose – are degraded via the primary metabolism of the fungus, while lignin degradation occurs during secondary metabolism (Eriksson et. al, 1990). Until there is limitation of an essential nutrient such as nitrogen, carbon, sulfate, or phosphate, it has been shown that the primary metabolism of the fungi occurs.

To degrade cellulose, white-rot fungi employ a variety of enzymes known as carbohydrate-active enzymes (CAZYS) which include glycosyl hydrolases (GHs) (Schilling et. al, 2020). Hydrolytic enzymes that are primarily involved include several types of endo-1,4- $\beta$ -glucanases (EC 3.2.1.4), which randomly attack and split the cellulose chain; exo-1,4- $\beta$ -

glucanase (EC 3.2.1.91), which releases cellobiose or glucose from the nonreducing end of the cellulose chain; and several types of 1,4- $\beta$ -glucosidases (EC 3.2.1.21) (cellobiase), which hydrolyzes the cellobiose to glucose (Eriksson et. al, 1990). This synergistic enzymatic attack of the endo-and-exo-1,4- $\beta$ -glucanases to degrade crystalline cellulose is unique to white-rot fungi. Despite the synergy however, the rate of depolymerization of the cellulose and hemicellulose is rather slow. This is believed to be the result of an intricate induction and repression system, where the cellulase enzymes are induced by the presence of cellobiose but repressed by small amounts of glucose (Eriksson et. al, 1990).

As for the lignin in the wood, white-rot fungi, particularly the basidiomycetes, are the best degraders of lignin among all known living microorganisms. Some ascomycetes also produce white-rot in hardwoods as well. (Eriksson et. al, 1990). An extensive amount of research has been conducted to understand how white-rot fungi selectively degrade lignin in biomass. It is believed that the lignolytic enzyme system of these fungi is triggered as a result of multiple physiological events, primarily nitrogen starvation. (Eriksson et. al, 1990). Carbohydrate or sulfur starvation are also believed to trigger the secondary metabolism. (Buswell et al., 1984). However, some white-rot fungi still manage to degrade lignin under conditions of nutrient sufficiency (Freer & Detroy, 1982). Lignin biodegradation is primarily oxidative and associated with a decrease in methoxyl content.

Unlike cellulose and hemicellulose, lignin is randomly oriented with variable linkages. To break this network of bonds, white-rot fungi target lignin with oxidative reactions via enzymes including peroxidases (PODs) and laccases, which unsheathe carbohydrates and allow for carbohydrate-active enzymes to access the cellulose and hemicellulose (Schilling et. al, 2020). Both types of enzymes are used in combination with associated enzymes and cofactors

(Daniel, 2016). However, there are large variations between different white-rot species in both the types and timing of the peroxidase enzymes that they produce, and the different types of white-rot that occur is believed to be a result of this. That said, the three main peroxidase enzymes that have been characterized are lignin peroxidase (EC1.11.1.14), manganese peroxidase (EC1.11.1.13), and versatile peroxidases (VPs EC 1.11.1.16) (Daniel, 2016). These enzymes work to degrade lignin in an assortment of ways, including cleavage of aryl-ether bonds between monomer lignin units, demethylation, and cleavage of the benzene rings to a fatty acid that can be fed into an acid cycle. Manganese peroxide has been found as the most prevalent and is therefore considered the most important (Hofrichter, 2002). All three enzymes are heme-containing glycoproteins that require  $H_2O_2$  as the oxidant (Martínez, 2002). The key to the peroxidase enzymes functioning is the presence of hydrogen peroxide ( $H_2O_2$ ), which can be stressed out of white-rot fungi through growth in low-nitrogen medium (Daniel, 2016).

Hydrogen peroxide oxidizes the peroxidase enzymes, producing an unstable intermediate that, when paired with a heme glycoprotein cofactor, leads to the degradation reactions. The lignin peroxidases (ligninases) are unique due to their higher oxidation potentials than most peroxidases (Aust, 1995). This allows them to oxidize non-phenolic lignin units through abstraction of an electron in the aromatic ring of lignin via activated oxygen, generating cationic radicals that lead to chemical decomposition (Hatakka, 2005). On the other hand, the manganese peroxidase enzymes oxidize phenolic lignin units to phenoxyl radicals, but also leads to decomposition (Hatakka, 2005). Laccase enzymes interact with a mediator compound, often a substrate secreted by the white-rot fungi, that allows them to oxidize and degrade both phenolic and non-phenolic lignin units through demethylation (Daniel, 2016). Of critical importance to the carbon cycle,

white-rot fungi decay lignin and carbohydrates present in both softwoods and hardwoods to CO<sub>2</sub> and H<sub>2</sub>O (Daniel, 2016).

## Brown-Rot Fungi

Relative to white-rot fungi, there are far fewer species of brown-rot fungi. By some estimates, brown-rot fungi comprise only 6-10% of the total number of wood-rotting fungi, and all the brown-rot fungi are basidiomycetes (Gilbertson, 1980). However, they are still exceptionally important ecologically and play critical roles in forest ecosystems. In relative terms (millions of years ago), brown-rot fungi developed from white-rot fungi who did possess extracellular phenol oxidases (Hibbett, 2001). Remarkably, this evolution appears to have occurred at parallel instances with each family of fungi that contains brown-rot fungi also containing white-rot fungi. Indeed, entirely unrelated families include brown-rot fungi, indicating that parallel, and not linked, evolution occurred across the fungal world (Hibbett, 2001). This also means that brown-rot fungi themselves are not closely related (Gilbertson, 1980). Rather, the traits that have come to define brown-rot fungi evolved repeatedly from different species of white-rot fungi (Hibbett, 2001). Further, evidence has been found that five of the families with brown-rot fungi are themselves polyphyletic.

A defining characteristic of brown-rot fungi is that they do not produce extracellular phenol oxidases, which provide white-rot fungi the ability to breakdown lignin (Gilbertson, 1980). Moreover, brown-rot fungi cannot degrade pure isolated cellulose. While the ability to break down lignin may appear to be an evolutionary advantage, therefore implying that white-rot fungi must have evolved from brown-rot species, the opposite is in fact true. Another key driver that distinguishes brown-rot fungi is that they predominantly have heterothallic and bipolar reproduction mechanisms, which represents a simplification of its mating system relative to the

tetrapolar reproduction system most common among white-rot fungi (Gilbertson, 1980). Whether this simplification of a reproduction system was linked to the reduction in variety of extracellular enzyme systems was related is unknown (Raper & Flexer, 1971). The reduction in extracellular enzymes however also coincided with the remarkable ability to selectively and rapidly remove cellulose components from wood (Hibbett, 2001).

Whereas white-rot fungi either selectively degrade lignin or simultaneously degrade the lignin, cellulose, and hemicellulose, brown-rot fungi are characterized by the aptitude to extensively degrade the cellulose and hemicellulose in wood while leaving the lignin intact but modified (Eriksson et. al, 1990). This is done through extensive depolymerization of the polysaccharides, particularly the hemicelluloses (Daniel, 2016). While some lignin is removed, the visual characteristics defining brown-rotted wood is an amorphous, crumbly residue that is composed primarily of lignin, which produces the signature brown color (Eriksson et. al, 1990). However, decay tests have revealed that brown-rot fungi cause substantially greater weight loss over identical periods of time and growth conditions (Gilbertson, 1980). As a result, wood decay by brown-rot fungi causes severe and rapid loss of strength in wood even at minimal loss of mass (Daniel, 2016).

While white-rot fungi are distinguished by an L/D ratio of greater than 0.8, brown-rot fungi cannot be distinguished from soft-rot fungi via the L/D ratio since both exhibit ratio below 0.8 (Worrall et. al, 1997). Instead, a characterization known as the wood dilute alkali solubility (DAS) is utilized, which has been found to increase far more during brown-rot than white or soft-rot due to carbohydrate depolymerization and some lignin modifications (Schilling et al., 2015). An aspect of brown-rot decay that contributes to this is that brown-rot fungi have been found to degrade hemicellulose dramatically faster than white-rot fungi (Kirk & Highley, 1973).

In one test, the xylan and mannan rates of loss were 55% and 44% greater for brown-rot fungi, respectively (Schilling et. al, 2020).

### Morphological & Chemical Changes

Brown-rot fungi cause decay by growing into the cell lumina of wood cells and launch an attack upon surrounding carbohydrates. During the decay process, the loss of cellulose and the attack upon the cell walls is both rapid and uniform (Daniel, 2016). In many ways, the decay process of brown-rot fungi serves as an antithesis to that of white-rot fungi that preferentially degrade lignin. The S3 layer of the secondary wall initially resists attack of brown-rot fungi, but the S2 layer, high in crystalline cellulose content, is degraded rapidly (Eriksson et. al, 1990). The greater the content of polysaccharides in a cell component, the greater the decomposition. Ultimately, however, crystalline cellulose is removed from all layers. The most remarkable feature of brown-rot fungi however in both softwoods and hardwoods is the extent of polysaccharide loss with a limited number of hyphae (Daniel, 2016). Moreover, research has found that degradation of cells adjacent to cells with hyphae also occurred, which suggests that an exceptionally diffusible enzyme is utilized to degrade (Eriksson et. al, 1990). Additional research has found that decay produced by brown-rot fungi occurs at a location distant from the fungal hyphae themselves, further supporting a diffusible enzyme system (Daniel, 2016).

Contrary to white-rot and soft-rot decay, the lignin type present in the wood and the difference between hardwoods and softwoods is unsubstantial, and both types of wood are degraded at equivalent rates (Daniel, 2016). This further implies that brown-rot fungi have a mechanism that can indiscriminately attack a substrate with a lignin barrier. Additionally, unlike in white-rot decomposed wood, the shape of the cell and the thickness of the cell wall are not noticeably changed (Eriksson et. al, 1990). Rather, with the removal of the cellulose and

hemicellulose, light microscopy has revealed that the secondary wall resembles something of a porous structure that then swells and contracts more readily with moisture fluctuations. When the decomposed wood shrinks, cracks and tiny cavities form which leads to an overall cubical appearance of the rotted wood (Eriksson et. al, 1990 – Liese 1970).

Despite their limited numbers, brown-rot fungi are still ecologically critical to forest biomes. Following advanced decay, the residue that is left is a brown, crumbly mass composed largely of lignin (Gilbertson, 1980). In some forests, research has found that up to 30% of the upper foot of soil volume was composed of brown-rot wood residue. Soils with high contents of brown-rot residue are known to have an increased water-holding capacity and provide preferable conditions for seed germination (Jurgenson et al., 1977). This residue has also been found to be a site of nitrogen fixation (Gilbertson, 1980). The reason for this is the presence of undegraded but chemically modified lignin in the fungal degraded wood residue that is then broken down by other microbes (Cornwell et. al, 2008).

### Wood-Degrading Enzyme System

As mentioned above, although brown-rot fungi evolved from white-rot fungi, they possess a smaller range of enzymes available for biomass degradation (Eastwood et al., 2011). Mainly, this reduction in enzymatic capabilities has to do with the inability to degrade lignin, though brown-rot fungi do substantially modify lignin through demethylation (Daniel, 2016). In brown-rot fungi, while the degree of polymerization of the cellulose is dramatically reduced and the hemicellulose almost entirely removed, there is little to no degradation of the lignin due to the capabilities of these fungi (Eriksson et. al, 1990). Interestingly, brown-rot fungi do not appear to even attack cellulose in the same manner as white-rot fungi as most brown-rot fungi do not possess the ability directly degrade pure, isolated cellulose, inferring that the process is far more

complex and that other wood components are a requirement for initiation of cellulolytic attack (Eriksson et. al, 1990).

The current theory is that this system is comprised of a nonenzymatic system to depolymerize cellulose through oxidative degradation followed by enzymatic breakdown in a two-phase attack (Daniel, 2016). Instead of using peroxidases to unsheathe carbohydrates, brown-rot fungi instead utilize reactive oxygen species (ROS) mechanisms that loosen the wood cell wall components as described and then apply a more limited set of glycosyl hydrolases (Zhang et. al, 2019). This system is based upon Fenton chemistry, where hydroxyl radicals produced by reacting  $\text{H}_2\text{O}_2$  with ferrous iron ( $\text{Fe}^{2+}$ ) oxidatively degrades cellulose (Daniel, 2016). For this to occur, the fungi require ferrous iron and  $\text{H}_2\text{O}_2$  to be present and substantial research has been conducted to verify the origins and involvement of the compounds. Of note is that the reaction is somehow activated from a distance, creating a remote attack away from the fungal hyphae that likely is necessary in order to protect the organism itself from the hydroxyl radicals (Daniel, 2016). Then, the brown-rot fungi produce endo-1,4- $\beta$ -glucanases, though no research has found production of exo-1,4- $\beta$ -glucanases (cellobiohydrolases). Despite the reduction in enzymatic capability, the rate of cellulose depolymerization is much faster than white-rot (Eriksson et. al, 1990).

## Soft-Rot Fungi

Soft-rot fungi receive their name from their unique ability to decay waterlogged wood, which was soft when touched (Daniel, 2016). Fungi that cause soft-rot decay include a diverse collection of microfungi and are taxonomically either ascomycetes or fungi imperfecti (Daniel, 2016). Unlike white and brown-rot fungi, whose aptitude for success under various environmental conditions are more difficult to pinpoint, wood in contact with excessive amounts

of moisture is directly at risk for attack by soft-rot fungi. That said, soft-rot fungi have also been found in some of the most extreme environments on Earth, including the Arctic and deserts (Blanchette, 2016). When dried, the decayed wood resembles that of brown-rotted wood with cracks and checks (Daniel, 2016). While soft-rot fungi cannot be identified by a singular test like the L/D ratio for white-rot fungi and the DAS for brown-rot fungi, combining results from the two can, as a low L/D ratio and low DAS value combine to represent soft-rot degradation (Schilling et. al, 2020). There is a general consensus that research of soft-rot fungi is lacking (Daniel, 2016).

### Morphological & Chemical Changes

Soft-rot fungi attack the cell walls of the wood, causing cavities in the S2 layer. While cavities are not a unique aspect of wood decay, the chain of cylindrical and diamond-shaped cavities that soft-rot fungi produce in the secondary cell wall is (Eriksson et. al, 1990). As seen with brown and white-rot fungi though, the specific morphology of the cavities varies wildly between both fungal species and different wood substrates (Eriksson et. al, 1990). On top of cavities, soft-rot fungi also tend to erode the cell wall, starting from the cell lumen and moving inward towards the middle lamella. It is these two types of attack that characterize soft-rot fungi. Fungal attack that leads to formation of cavities within the cell wall is referred to as Type I attack, while Type II attack involves the complete erosion of the secondary wall layers directly beneath the hyphae (Daniel, 2016). Unlike white-rot fungi who exhibit either simultaneous or lignin selective decay, soft-rot fungi may produce either Type I attack or Type I and Type II attack. Fungi that only utilize Type II attack however are not considered soft-rot fungi, as the resulting wood is more characteristic of white-rot degradation (Eriksson et. al, 1990).

Of note, most soft-rot fungi that exhibit both Type I and Type II attack in hardwood substrates produce only Type I attack in softwood substrates. Thus, cavity formation is the most distinct, reliable characteristics of soft-rot fungi and is the best trait from which to identify them (Eriksson et. al, 1990). While there are isolated instances of reports of brown and white-rot fungi producing cavities in the S2 layer, it is believed that localized collapse of degraded S2 layers may be the reason for these phenomena, and that cavitation is only characteristic of soft-rot fungi (Daniel, 2016). Wood substrate also plays an influential role in the rate and extent of decay by soft-rot fungi as soft-rot decay is significantly impacted by the lignin type present and its concentration, unlike brown-rot, and to an even greater extent than white-rot (Daniel, 2016). Given that both Type I and Type II attack is seen in hardwood substrates, it follows that hardwoods are generally attacked to a greater extent as softwoods (Eriksson et. al, 1990). Further, in hardwoods, carbohydrates are removed faster than lignin, while in softwoods, lignin is removed faster than the cellulose or hemicellulose (Eriksson et. al, 1990). On a standard time basis, research has found that both the rate of decay and the degradative extent of soft-rot fungi on softwood substrates is minimal compared to that of white and brown-rot fungi (Eriksson et. al, 1990). Mass loss is also highly variable among soft-rot fungi.

There are further differences in the way that soft-rot fungi colonize different types of wood. In hardwoods, soft-rot fungi first colonize the rays and vessels, and then the hyphae grow into the fiber lumina. Meanwhile, in softwoods, the hyphae colonize ray parenchyma cells and move from the rays into the lumina of tracheids (Eriksson et. al, 1990 – Liese 1970). Regardless of starting position however, the hyphae spreads by boring into the secondary cell wall and into the S2 layer, where they then proceed to align with the direction of the cellulose microfibrils. Upon extending into the S2 layer, the hyphae then halt their growth and release enzymes along

the length of the hyphae, leading to dissolution of the cell wall from the inside out (Hale & Eaton, 1985). As the cavity volume increases, new hyphae are extended outward into new cells, leading to the characteristic series of cylindrical and diamond-shaped cavities. As decay progresses, the cavities grow larger, but only in the radial direction of the cylindrical cavities, not in length (Daniel, 2016). This is believed to be a result of the way the hyphae align with the microfibrils, which indicates that the release of enzymes is ineffective longitudinally into the microfibrils. As decay progresses, many cavities merge, yielding substantial areas of decay. Again, the species of fungi, growth conditions, and substrate all dramatically influence cavity formation in ways that are not entirely understood (Hale & Eaton, 1985).

The most likely difference between softwood and hardwood that influences the rate of decay and ability of soft-rot fungi is the quantity of lignin in the wood. Across the board, wood substrates with low lignin content are far more susceptible to soft-rot fungal degradation than those with high concentrations of lignin (Eriksson et. al, 1990). Though not confirmed, it is believed that the lignin inhibits soft-rot enzymatic action in some manner, with Guaiacyl components of softwood lignin presenting the greatest obstacle to degradation by soft-rot fungi. (Eriksson et. al, 1990). Some research has found that in substrates with S2 layers with unusually high lignin content, a cavity in the shape of a half-moon is produced rather than a full cylinder, potentially because of the lignin barrier.

### Wood-Degrading Enzyme System

Relative to white and brown-rot fungi, soft-rot fungi utilize wood degradation mechanisms that are far less studied. In general terms, soft-rot fungi attack wood via cavitation and cell wall erosion and their main priority is the polysaccharide sugars, often leaving lignin mostly undigested (Blanchette et. al, 2010). Currently, it is assumed that the enzymes used in the

formation of cavities do not differ from the enzymes used in cell wall erosion. Given the alignment of the soft-rot fungi hyphae with the cellulose microfibrils, it is theorized that this alignment induces the secretion of cellulases that then degrade the wood cell walls (Daniel, 2016). While the ability to produce all the necessary extracellular enzymes for crystalline cellulose degradation varies among soft-rot fungi, limited research has found that the cellulase enzymes employed by soft-rot fungi are very similar to those of white-rot fungi (Eriksson et. al, 1990). Though with slight variations, soft-rot fungi are documented in having endoglucanases, exoglucanases/cellobiohydrolases, 1,4- $\beta$ -glucosidases (Eriksson et. al, 1990). For soft-rot fungi that are incapable of producing the full set of enzymes, only the amorphous cellulose can be degraded.

While specific soft-rot fungi have been studied and found to possess the capability to degrade various components of biomass, there exists a lack of evidence to provide a general understanding of how they degrade wood. The ability to remove cellulose and hemicellulose preferentially and leave lignin in cavities indicates the utilization of an enzymatic system including cellulases and hemicellulases but a more specific understanding remains elusive (Daniel, 2016).

## Global Distribution of Rot Type

In the characterization of the vast realm of fungi, there have been attempts to draw correlations between species such that they may be neatly categorized and separated into appropriate sections based on various key traits. The use of brown-rot, white-rot, and soft-rot is the most basic and readily available example. But as has been seen through the discussion of these types of fungi, variability often outpaces predictability, and oftentimes trends are riddled with exceptions. Even the brown/white-rot classification has come under fire in the past decade

with the proposition of “gray rot,” and the idea has garnered considerable traction in recent years (Riley et. al, 2014). This has made classification of fungi exceptionally difficult, and the analysis of the geographic distribution of the different types of wood-decay fungi an ongoing process. There are several theories behind the geographic distribution of fungi that will be explored in this section, all of which have their pros and cons, and of course, exceptions.

While the following theories are debated, one aspect remains widely agreed upon – that species of fungi are more widely distributed geographically than species of higher plants, presumably due to fungi’s unique capability of long-range, aerially spread spores (Gilbertson, 1980). To compound that, fungi are found in nearly every biome imaginable – from the Mohave desert to the arctic tundra. Further, the range of a fungi is often quite different than any single host, further complicating matters, even though many fungi are named after their major tree host (Bisby, 1943).

## Geography

One of the initial attempts to develop a theory of “mycogeography” was by Gilbertson, who believed that while much of the world was still “terrae incognitae fungorum,” or unknown territory in the study of fungi, there existed enough information on northern hemisphere fungi to adopt general trends (Gilbertson, 1980). These initial trends were based almost entirely upon latitudinal geography, with brown-rot fungi dominating higher latitudes above the tropic of cancer, white-rot fungi dominating at lower latitudes, and soft-rot fungi occupying extremes. Essentially, countries and regions at similar latitudes around the globe should possess similar species of fungi while regions at different latitudes should expect variation in fungal species.

Evidence for this theory includes observations that 60% of the fungi reported in Manitoba also occur in Europe, and 70% of fruiting-body fungi in North America also occur in Europe

(Gilbertson, 1980). Indeed, a comprehensive meta-analysis by Gilbertson found countries that were latitudinally similar with the United States shared a high percentage of fungal species. Sweden shared 90% of the wood-rotting fungal species with the United States, Finland shared 87%, Norway shared 85% Northern Europe shared 84%, and Russia shared 70% (Gilbertson, 1980). Meanwhile, countries in the southern hemisphere including Australia and New Zealand were found to share only 38% and 30% of the wood-rotting species with the United States, respectively. Further, when there were exceptions to this rule, Gilbertson claims these can be accounted for by taking altitude into account. For example, although the Himalayas are at a latitude of 30 degrees N and the Rocky Mountains stretch along a range of 35-55 degrees N, 72% of the species in the Himalayas are found in the Rocky Mountains (Gilbertson, 1980).

One potential counterargument to this theory is that as European colonization occurred fungal species were inadvertently spread; however, Gilbertson dismisses this idea with an explanation that all of the North American wood-rotting fungi are endemic and that none have been introduced as a result of human activity (Gilbertson, 1980). Given the data available to Gilbertson at the time, the correlation between fungal rot type and latitude makes sense; however, it remains just that – a correlation – and the world's growing database of fungal species continues to find exceptions. Moreover, latitude itself has nothing to do with fungal growth or decay, indicating there are perhaps more relevant characteristics at play when determining the distribution of fungi.

But correlation of latitude to fungal distribution does not imply causation. The variation of fungal species distribution within North America itself is also very high, to the point that Gilbertson himself says that “certainly it is impossible to make any generalized statement on the subject” (Gilbertson, 1980). A better place to start such a search regarding the global distribution

of fungi would be to look at what certain requirements fungi have for growth and survival. If different areas and different fungi have different conditions and requirements, that would potentially explain distributions. In general, wood-rotting fungi have six major growth needs (Zabel, 2020). First, there must be free water on the surfaces of the wood cell lumina. Second, the atmospheric oxygen levels must be relatively low. Third, the climate should lay in a favorable temperature range, with most wood-rotting fungi's optima temperature between 15 and 40 degrees Celsius. Fourth, the type of wood substrate must provide the necessary and specific materials for energy and metabolites. Fifth, the substrate should lay in the favorable pH range of 3 to 6. Last of all, chemical growth factors such as nitrogen content and other essential elements must exist at certain minimum thresholds.

### Climate: Moisture and Temperature

A region's climate has pertinent impacts on key environmental factors that affect fungal growth conditions, particularly the temperature and precipitation level, which may impact the moisture content of wood. This may have led Gilbertson to draw a correlation between latitude and fungal distribution because, to an extent, latitude is also correlated to the climate. Moisture content of wood is a critical characteristic for decay because wood-rotting fungi are unable to metabolize wood if its moisture content is below the fiber saturation point, which is typically around 25% (Zabel, 2020). Thus, for wood-decaying fungi, the minimum moisture content level before decay can be initiated ranges from 25-32%, or slightly above the fiber-saturation point of the wood (Zabel, 2020). At this point, free water becomes available on the wood cell walls' surface, which the fungi then use as the medium to diffuse the enzymes from their hyphae (Gilbertson, 1980). For perspective, keeping commercial wood below a threshold of 20% moisture content is the standard level for preventing decay (Cartwright & Findlay, 1946).

On the other side of the spectrum, most fungi are also unable to grow on wood that is saturated with water, as fungi are aerobic organisms that have minimum requirements for respiration (Zabel, 2020). As moisture content increases in wood, the air in the cell lumina is replaced by water. Therefore, the void volume (size) of the cell lumina, which is inversely related to the specific gravity of the wood, plays an important role in establishing upper thresholds of decay. The denser the wood, the lower the maximum moisture level. However, one type of fungi – soft-rot fungi – manage to not only survive but thrive under these high moisture content environments (Zabel, 2020). Some soft-rot fungi have been found to be able to degrade wood submerged in the ocean or in the nearly saturated environments on the baffles of water towers (Zabel, 2020).

Overall, the optimum moisture content level for wood-rotting fungi falls in the wide range of 40-80% (Scheffer, 1973). That said, the optimum moisture content variability between fungi is known to be high and further investigation of the trait may help to explain some of the substrate and distribution correlations between fungi, wood species, and location (Zabel, 2020). Though evidence is currently acknowledged as limited, it is believed that white-rot fungi require higher moisture content levels than brown-rot fungi (Highley & Scheffer, 1970). Brown-rot fungi have also exhibited a tolerance to regions of lower rainfall, which typically means that less of the year is suitable for wood-rotting fungi to grow (Gilbertson, 1980). This may potentially explain why white-rot fungi become more prevalent on trees in wetter regions, which are often at lower latitudes, compared to the dominance of brown-rot fungi on trees in drier regions, which are often at higher latitudes (Zabel, 2020).

Similarly, general temperature trends do vary north to south with latitude, but they vary more specifically with climate. Every fungus possesses its own three cardinal growth

temperatures – a minimum where growth begins, an optimum where the highest rate of growth occurs, and a maximum where growth ceases (Zabel, 2020). In general, the optimum temperature tends to skew towards the maximum. While there are fungi that can grow below 0 degrees Celsius, known as psychrophiles, and other fungi that thrive at extreme temperatures, known as thermophiles, wood-rotting fungi are typically referred to as mesophilic because their optimum temperature range is 15-40 degrees Celsius (Zabel, 2020).

Like moisture content levels, there is substantial interest in how temperature requirements impact the distribution and substrate preference of wood-decay fungi. Some fungal species' basidiocarps only develop under snow, such as *Tryomyces leucospongia*, *Phaeolus alboluteus*, and *Lentinellus montanus* and most interestingly, all them are brown-rot fungi (Gilbertson, 1980). The rationale behind these phenomena is that brown-rot fungi more efficiently extract energy from wood and are therefore able to grow and reproduce in shorter lengths of time (Gilbertson, 1980). Overall, however, there is an insufficient quantity of data available to draw definitive trends (Zabel, 2020). Some research though has found that temperature requirements may vary widely between species and even vary dramatically within a species (Esllyn, 1970). Based on limited available data from a handful of fungal species, both brown-rot fungi, such as *Serpula lacrymans* and *Phaeolus schweinitzii*, and white-rot fungi, such as *Phellinus pini* and *Heterobasidion annosum*, have optimal growth temperatures below 25 degrees Celsius. Meanwhile, brown-rot fungi, such as *Gloeophyllum striatum* and white-rot fungi, such as *Lentinus strigosus* and *Trametes hirsute* have optimal growth temperatures of above 34 degrees Celsius.

Similar to moisture content, soft-rot fungi again stand out among the wood-rotting fungi for their greater tolerance of higher optimal temperatures and tolerances relative to brown and

white-rot fungi (Zabel, 2020). In experiments on chip piles, soft-rot fungi have shown considerable heat tolerances, being the only wood-decay fungi to survive at temperatures greater than 65 degrees Celsius for up to 72 hours (Hulme & Stranks, 1976). However, many soft-rot fungi have also been found to exhibit higher growth rates at lower temperatures similar to brown and white-rot fungi, which leads to the question of whether soft-rot fungi merely exist in the ecological niche of wood-rotting at higher temperatures even if it is not in their optimal range (Morrell, 1981).

Using climate to distinguish fungi has an extensive history in the field, with the widely utilized Scheffer Index – also known as the Climate Index Value – to estimate decay hazard (Scheffer, 1971). This formula, based upon standard U.S. Weather Bureau summaries, creates an index of the potential the climate has to increase the risk of and promote decay of wood structures (Scheffer, 1971). The Scheffer index synthesizes experimental data of decay rates of fungi, climatological data from the US Weather Bureau, and applies a range of 0 to 100 for the potential for decay (Scheffer, 1971). To simplify the formula, only precipitation and temperature data are considered.

Though originally developed for the United States, Scheffer Index maps have also been made for Canada, Australia, China, Spain, and South Korea (Carll, 2009). For the United States, it was found that the southeast, particularly Florida, possesses the highest decay index in the range of 70-100, stretching from Louisiana up to Virginia (Scheffer, 1971). Other states east of the Mississippi River (Northeast to the Midwest) as well as Oklahoma up to South Dakota display decay indexes in the middle range of 30-70. The rest of the country – the west – has decay indexes of 0-30 aside from a small strip along the Pacific Ocean coastline stretching from Washington down to Oregon that varies dramatically.

While the Scheffer Index is certainly helpful in quantifying the decay risk of wood on a geographic basis, it does not distinguish between rot type – soft, white, or brown – which as previously noted have dramatically different impacts on the state of decayed wood. Additionally, the map is primarily focused on aboveground commercial wood structures. While the conditions in aboveground commercial wood may be similar to that of the climate, both moisture and temperature conditions in trees can vary dramatically, for example depending upon the density of the wood substrate (Zabel, 2020). In fact, an important distinction has been made that specifically wood temperature and wood moisture content are more important to decay risk rather than the climate conditions (Carll, 2009 – Brischke and Rapp 2008). Last of all, climate changes, and later research has found that the Scheffer Index of specific cities may vary dramatically between decades (Carll, 2009). Lastly, many have concluded that recorded climate-based temperatures are not a useful consideration when looking at the distribution of wood-decay fungi (Zabel, 2020). As for moisture, the reason the Scheffer Index may be misleading is the apparatus fungi use to tolerate varying levels of moisture content – pseudosclerotial plates that protect the exterior portions of the decaying wood and regulate moisture content. Further, this apparatus is non-unique to a specific group of wood-rotting fungi. Indeed, while soft-rot fungi in rainforests display a remarkable tolerance for high moisture environments by limiting the entrance of excess water, the same apparatus appears to be used by brown-rot fungi to restrict the loss of water in drier climates (Blanchette, 1991).

When it comes to the distribution of soft-rot fungi meanwhile, they truly inhabit areas of extremes – the arctic, deserts, and the tropics. This remarkable diversity may stem from the diversity of soft-rot fungi as a classification. It may also explain the lack of research and information on the group, considering the associated difficulties in studying them. In Arctic

driftwood, soft-rot fungi from the Ascomycota dominate, somehow overcoming both the extreme cold and marine environment (Blanchette, 2016). These arctic soft-rot fungi are mainly generalists – meaning they can degrade any type of wood. Different fungal communities were found between Greenland, Iceland, and Siberia, and many of the species were mutually exclusive to their region.

In another remarkable example, soft-rot fungi, also ascomycetes, were found on the expedition huts of used in the expeditions to explore Antarctica between 1901 and 1911 despite being located in one of the coldest and driest locations on Earth (Blanchette, 2004). While the extent of the decay was very limited and deemed to not be of concern to the structural integrity of the huts, the isolated species did cause extensive soft-rot decay when tested on wood samples in laboratories (Blanchette, 2004). Clearly, soft-rot can occur when wood is either very wet or very dry. Next to no basidiomycetes survive in the arctic; however, as the permafrost layer continues to melt and the arctic warms, there is concern that the range of particularly white-rot fungi could expand, influencing the carbon cycle and increasing the quantity of greenhouse gases released from decayed wood.

### Substrate Relationship

Perhaps the most prominent theory at present is that fungal distributions may be predicted based upon the distribution of hosts (Gilbertson, 1980). For example, conifers and other softwood trees typically occupy boreal regions or high elevation habitats because they can withstand the shorter growing season and more severe temperature restrictions. It is also true, as mentioned earlier, that brown-rot fungi are better suited for colder and drier habitats than white-rot fungi because of their greater energy extraction efficiency and faster reproduction cycles. So,

the question becomes whether brown-rot fungi simply best exist in the same climate as softwoods, or do they truly prefer softwoods.

In his initial theory regarding the correlation between latitude and rot type, Gilbertson also stated that there was an “obvious relationship” between brown-rot fungi and the preference for conifer substrates, which are classified as softwoods (Gilbertson, 1980). One survey found that in the Polyporaceae family of the basidiomycetes, which includes 71 species of brown-rot fungi, 85%, or 60 of the 71 species are primarily found on conifers (Lowe, 1966). Such a strong preference was believed to have been found in other groups as well. According to data available at the time, brown-rot fungi in the families of Coniophoraceae, Corticiaceae, Agaricales and Stereaceae were also found to primarily attack conifers as well. By looking at seven conifer species, it was found that of the 206 fungi present, 24% were brown-rot fungi, compared to seven species of hardwood, where of the 114 species, 7% were brown-rot fungi (Gilbertson, 1980). An important note to make however is that while it is true that brown-rot fungi prefer softwoods over hardwoods, that does not mean they are the only fungi to attack softwoods, and that brown-rot fungi do not attack hardwoods. In a comprehensive review of fungal species present on various species of wood, Gilbertson found evidence to support such a relationship but hardly enough to definitively distinguish the two substrates and two types of fungi. Softwood substrates including Ponderosa Pine, Western true firs, Spruces, Western Hemlocks, and Douglas Fir have all had 200+ species of wood-rotting fungi identified on them. Of those 200+ species, between 18.8% to 23.3% of them were brown-rot fungi. When it comes to hardwoods including Aspen, Western Alders, Western Maples, and Western Birches, all had 100+ species of wood-rotting fungi identified on them; however, only 7.0% to 11.8% of those identified fungi were brown-rot fungi. While that indicates that brown-rot fungi are half as likely to be found on

hardwoods than on softwoods, it does mean that upwards of 25 different brown-rot species attack Aspen alone (Gilbertson, 1980).

Moreover, while this data appeared to draw an interesting connection between brown-rot fungi and conifer substrates, it says little about the preference of white-rot fungi. The earliest white-rot fungi, found in fossil records as far back as the Triassic period, appeared to attack softwood gymnosperms (Blanchette, 1991). Early gymnosperms were characterized by remarkably high lignin contents and large extractive concentrations, which would have made lignin-capable degradative mechanisms a necessity (Blanchette, 1991). Later research seemed to confirm though that most white-rot fungi species prefer angiosperms (hardwoods), but a significant number also degrade gymnosperms (softwoods) and that the diversity among different species is significant (Blanchette, 1991). For example, a well-known white-rot fungus, *Phellinus pini*, causes extensive decay in conifers across the globe (Blanchette, 1991).

The correlation between brown-rot fungi and conifer substrates (as well as a bipolar mating system) was tested for a causal relationship by Hibbett at the turn of the 21<sup>st</sup> century (Hibbett, 2001). In this study, it was found that there was a statistically significant correlation between brown-rot – as a trait rather than the fungi itself - and exclusive decay of conifer substrates (Hibbett, 2001). Further, it was theorized that the evolution of brown-rot fungi has prompted repeated shifts to conifer substrate specialization (Hibbett, 2001). While Gilbertson reasoned that brown-rot fungi decay wood more rapidly, providing them a fitness advantage over white-rot fungi in conifer-dominated forests, Hibbett theorized that softwood trees likely possess some physical or chemical property that makes them more accessible to brown-rot fungi (Hibbett, 2001).

However, Hibbett did acknowledge that the statistical significance of the correlation, which would imply causation, was dependent upon the definition of conifer exclusivity that was set. The study also acknowledged that using a different statistical method led to ambiguous results, and that brown-rot fungi specifically from the polyporaceae family were overrepresented in the sample size. Further, it was found that conifer exclusivity has evolved five or six times within taxonomically linked groups of white-rot fungi as well (Hibbett, 2001). Therefore, while brown-rot fungi may have a greater tendency to exhibit conifer exclusivity than white-rot fungi, it is not an exclusive trait (Hibbett, 2001). Of note, only 130 species were analyzed.

More recently, in the most comprehensive analysis to date, a study that analyzed 1157 wood-decaying fungal species using a statistical software package in R found that brown-rot fungi are either generalists or gymnosperm specialists while white-rot fungi are predominantly either generalists or angiosperm specialists (Krah et. al, 2018). Interestingly, the analysis also found exceptionally high transition rates between generalism and host specialization in both white and brown-rot fungi species. However, the majority of brown-rot transitions were gymnosperm specialists to generalists while the majority of white-rot transitions were from generalists to angiosperm specialists (Krah et. al, 2018). Of the 1157 species studied, 126 were brown-rot fungi and 1031 were white-rot fungi. Each fungus was deemed a “specialist” if more than 90% of its host species were either angiosperm or gymnosperm (Krah et. al, 2018). With that threshold, it was found that 205 were gymnosperm specialists, 565 were angiosperm specialists, and 387 were generalists. It was found that 51% of white-rot species are specialized to angiosperm hosts, whereas 27% of brown-rot fungi are specialized on gymnosperm hosts. Interestingly, white and brown-rot fungi do not dramatically differ in their average number of host species, though brown-rot fungi have a larger average host range on gymnosperms and

white-rot fungi have a larger average host range on angiosperms (Krah et. al, 2018). By further breaking brown-rot fungi into five taxonomic clades, it was found that only two of the five consist mainly of gymnosperm specialists, while the rest are comprised primarily of generalists (Krah et. al, 2018).

Clearly, this contradicts Gilbertson's observation that 85% of brown-rot fungi occur primarily on conifers, and Krah et. al goes as far as to state in their conclusion that their results directly challenge the view that brown-rot fungi are primarily gymnosperm specialists. Krah et. al rationalizes this variation due to the limited scope of Gilbertson's analysis and explain that increasing the sampling size of species must continue to establish more trends. The study also shows the remarkable computational ability of software to analyze substantial quantities of data and provide a dramatic increase in the number of species studied.

A potential theory for the more recent specialization of white-rot fungi on angiosperms is the diversification of the angiosperms since the Cretaceous period (Krah et. al, 2018). Further, it is interesting to note that most white-rot fungi preferentially degrade syringyl (S) units of lignin while guaiacyl (G) lignin are more resistant (Hatakka, 2005). Thus, because hardwood species contain both S-lignin and G-lignin, as opposed to softwood species, which contain only G-lignin, white-rot fungi would theoretically be able to more effectively degrade hardwoods. That said, a few white-rot fungi do appear to at least possess the ability to degrade G-lignin as well.

The wood substrate also appears to play a role in which type of white-rot fungi dominates, with simultaneous types of white-rot occurring on trees with high lignin contents and selective lignin types of white-rot attacking only trees with lower levels of lignin (Blanchette, 1991). While some species such as *Phellinus pini* and *Ganoderma tsugae* always cause selective delignification, the anatomical and chemical composition of the wood appears has been found to

influence the distribution and type of rot (Blanchette, 1991). However, using this correlation to produce geographic ranges of specific fungi is inhibited by the ability of some white-rot fungi to interchangeably utilize selective or simultaneous decay methods depending upon what substrate they are attacking and under different environmental conditions (Otjen et. al, 1987).

### Chemical Growth Factors and pH

A couple of final and more recent attempts to correlate the distribution of fungal species to a specific factor related to the different types of decay has explored the impact of chemical growth factors such as Nitrogen and Phosphorous. Particular interest surrounds the nitrogen content of wood, which is known to play a crucial role in wood decay (Blanchette, 1991). Like other organisms, wood-rotting fungi require substantial quantities of nitrogen for a wide range of uses including protein synthesis, enzymes, and to produce chitin – a component in the fungi's hyphal cell wall (Zabel, 2020). Remarkably, even though nitrogen content in wood is only 0.03% to 1%, fungi manage to obtain their nitrogen needs from what is available in the wood (Zabel, 2020).

It was initially theorized that fungi manage to fixate nitrogen from the atmosphere, but this has since been disproven (Zabel, 2020). Instead, a far more complex system of nitrogen conservation by hyphal autolysis – the self-digestion of old hyphae - and nitrogen recycling has been found to exist (Cowling, 1970). How this may relate to type of decay is the importance of nitrogen conservation in the close regulation of cellulose-degrading enzymes (Zabel, 2020). In one study of white-rot fungi, it was found that lignin degradation is stimulated by low nitrogen levels while polysaccharide degradation is promoted by high nitrogen levels (Blanchette, 1991). In other experiment in the forests of Southern Chile, low nitrogen content of wood was found to be a major factor that led to complete delignification of the wood, while trees with high nitrogen

concentrations had increased polysaccharide breakdown (Blanchette, 1991). A nonuniform distribution of nitrogen within wood has also been used as a potential explanation for alternating areas of selective and simultaneous lignin degradation for some mottled rots in temperate forests (Eriksson et. al, 1990). Ongoing research is taking a closer look at whether wood decay distribution is driven by nitrogen and phosphorous nutrient availability, with the hypothesis being that brown-rot fungi utilize nutrients more efficiently, thus requiring less nitrogen to decompose wood.

Though the pH of soil and wood substrates undoubtedly plays a role in fungal decay, research on the correlation between pH and distribution is nonexistent, with any research relating to pH mainly focusing in on the performance of fungal enzymes at various pH levels. That said, it is known that wood-rotting fungi exhibit optimal growth rates in the pH range of 3 to 6 and that brown-rot fungi have been found to possess the lowest optimal pH of around 3 to 3.5 while white-rot fungi tend to prefer a pH range of 4 to 4.5 (Zabel, 2020). Soft-rot fungi display a tolerance to a wide range of pH conditions (Zabel, 2020). Future research may explore how soil or soil substrate pH plays into the distribution of rot type.

## Conclusion

Fungi are the dominant forest decomposers and are responsible for either recycling or releasing the Earth's largest of storage of aboveground carbon in wood. Fungi with the ability to degrade wood are characterized as white-rot, brown-rot, or soft-rot fungi depending on what type of decay they produce. White-rot fungi enzymatically decompose lignin either selectively or simultaneously to access the energy-rich wood carbohydrates of cellulose and hemicellulose, while brown-rot fungi use a non-enzymatic attack using Fenton chemistry that depolymerizes the wood carbohydrates while leaving the lignin only partially modified. Understanding of soft-rot

fungi is limited due to the diversity and lack of research but are believed to enzymatically create cavities in wood and possess an ability to degrade lignin.

There have been many theories regarding the global distribution of fungi based upon their decay type, which is important to monitor in order to understand whether carbon stored in wood is being released to the atmosphere (white-rot fungi) or remaining and enriching soil (brown-rot fungi). The earliest theories of fungal rot type distribution were proposed by Gilbertson and correlated latitude to rot type. Brown-rot fungi dominated in northern latitudes, white-rot in lower latitudes, and soft-rot in the extremes – the tropics, the arctic, and deserts. As research has advanced, new theories and attempted correlations focused on correlating factors directly related to fungal growth to distribution. The use of climate as a predictor of decay risk is widely used via the Scheffer Index, though the applicability of this scale to sound wood is questionable. This is because fungi possess the ability to regulate moisture content in wood to a suitable level, and that the temperature and moisture content in wood may differ from the climate due to wood density and other factors.

The prevailing theory at present is that whether the substrate is either hardwood (angiosperm) or softwood (gymnosperm/conifer) most accurately predicts what type of wood-decaying fungi will be present. Research from Gilbertson, Blanchette, and Hibbett all supported the relationship that brown-rot fungi selectively preferred softwoods and white-rot fungi generally preferred hardwoods. An analysis by Krah et. al of 1157 species however has since found that while many brown and white-rot fungi are generalists and able to degrade both hardwoods and softwoods. Recently, research has begun to look at whether chemical growth factors such as nitrogen or phosphorous content of the soil or wood better correlate and predict fungal rot type dominance. Ultimately, a single, simple method to determine the distribution of

the different types of wood-decaying fungi remains elusive, and while many general trends have been established, counterexamples merely leave these as patterns without predictability.

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