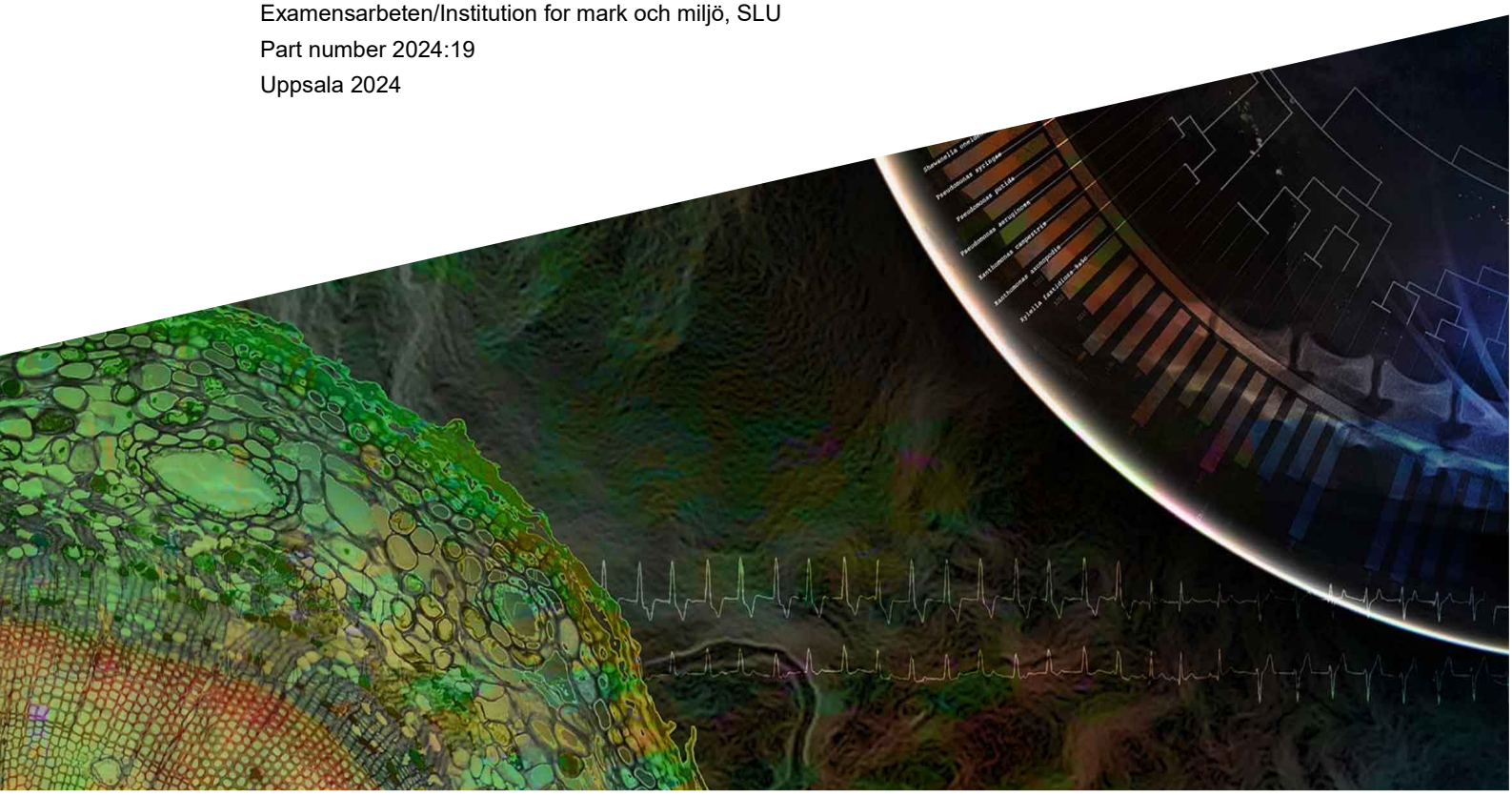
Do white rot fungi utilize carbon derived from lignin?

Lisa Larsson

Independent project, 15 credits
Swedish University of Agricultural Sciences, SLU
Department of Soil and Environment
Examensarbeten/Institution for mark och miljö, SLU
Part number 2024:19
Uppsala 2024



Do white rot fungi utilize carbon derived from lignin?

A literature review about white rot saprotrophic fungi and their role in decomposition within the boreal forest ecosystem

Lisa Larsson, Swedish University of Agricultural Sciences, Department of Soil and Environment

Supervisor:	**Erica Packard, Swedish University of Agricultural Sciences, Department of Soil & Environment**
Examiner:	Jon Petter Gustafsson**,** Swedish University of Agricultural Sciences, Department of Soil & Environment

Credits:	15
Level:	G2E
Course title:	Bachelor thesis in Biology
Course code:	EX0894
Programme/education:	Agriculture programme – Soil/Plant
Course coordinating dept:	Department of Soil & Environment
Year of publication:	2024
Place of publication:	Uppsala
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	white rot, saprotrophic fungi, fate of carbon in lignin, boreal forest, cellulose, hemicellulose, lignin, hydrolytic enzymes, oxidative enzymes, metabolism, intracellular pathways, extracellular pathways, carbon cycle, decomposition, decomposition rate, Mineralization/Immobilization, white rot preference for carbon source, lignin toxicity, lignin derivatives toxicity, white rot can utilize lignin as a carbon source

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Soil and Environment

Abstract

This study aims to investigate what happens to the carbon in lignin when it is broken down by white rot saprotrophic fungi in boreal forest ecosystems. Lignin is a complex and durable material that plays a significant role in storing carbon in these ecosystems. While there has been extensive research on how white rot fungi break down lignin, there is still debate on whether they can use lignin as a source of carbon. The goal is to gain a better understanding of the complicated processes and environmental impacts of transforming carbon through lignin degradation in boreal forests by conducting a literature review on the interaction between saprotrophic fungi and lignin. Additionally, an experiment was conducted to test how coniferyl alcohol a subunit of lignin is modified.

This research highlights the complexity of lignin degradation and the role of fungi in carbon cycling within boreal forests. Understanding these processes is crucial for several reasons, for example it can provide insights into the ecological functions of fungi and their interactions with other organisms in the forest ecosystem.

The literature review revealed that while significant progress has been made in understanding the biochemical pathways and enzymes involved in lignin breakdown, there are still many unknowns regarding the ecological and environmental factors that influence these processes.

The experimental component of the study showed how difficult it can be to conduct a simpler experiment. And that even in a controlled environment things don't always go as planned.

Based on the results, gaps were identified, and the question needs further research. We need to dig deeper into how fungi metabolize lignin-derived carbon inside their cells. Understanding these processes is key to seeing how carbon cycles through forest ecosystems. If fungi can not only break down lignin but also use its carbon for growth, it could greatly affect soil carbon dynamics and shift their ecological roles. This highlights the need for advanced genetic tools and protocols to study these interactions in natural settings.

Keywords: white rot, saprotrophic fungi, fate of carbon in lignin, boreal forest, cellulose, hemicellulose, lignin, hydrolytic enzymes, oxidative enzymes, metabolism, intracellular pathways, extracellular pathways, carbon cycle, decomposition, decomposition rate, Mineralization/Immobilization, white rot preference for carbon source, lignin toxicity, lignin derivatives toxicity, white rot can utilize lignin as a carbon source

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Abbreviations

SOM	Soil organic matter
LiP	Lignin peroxidases
MnP	Manganese peroxidases
CUE	Carbon use efficiency

1. Introduction

Lignin plays a significant role in the carbon storage of boreal forests (Thevenot et al., 2010). The process of its decomposition involves saprotrophic fungi that create white rot (Lundell, 2010). White rot fungi are characterized by the "white rot" phenotype, which refers to the white stringy residue they leave behind after degradation (Ahmed et al., 2013; Gonzalez et al., 2020; Martínez et al., 2005). This phenotype contrasts with brown rot fungi, which only degrades cellulose and hemicellulose while leaving most lignin behind (Dashora et al., 2023). White rot fungi are the only known organisms that can fully degrade lignin (Campbell et al., 2020; Floudas., 2021; Agosin et al., 1985; Lundell et al., 2010). Without this capability, a lot of carbon would remain inaccessible.

Lignin is a complex aromatic polymer present in the cell walls of vascular plants, providing structural support and defense against microbial intrusion (Martínez et al., 2005). Unlike cellulose and hemicellulose, which primarily consist of sugar molecules and are relatively easy to degrade, lignin is a complex and more resilient material (Eriksson et al., 2011; Lundell, 2010; Ahmed et al., 2013). The intricate three-dimensional configuration of lignin, which is rich in phenolic compounds, is responsible for its robustness and rigidity (Floudas, 2012; Martínez et al., 2005). Some compounds in lignin can also be toxic to some microorganisms (Martínez et al., 2005). Understanding what happens to carbon after it's broken down by white rot fungi is crucial for grasping the carbon cycle, nutrient recycling, and overall ecosystem functioning.

The primary objective of this study is to investigate the interaction between white rot fungi and lignin, shedding light on the complex mechanisms and environmental consequences linked to the transformation of carbon stemming from lignin degradation in boreal forests. The study will blend ecological and microbiological viewpoints to give us important insights into the often overlooked but essential role of carbon dynamics in boreal forests. By conducting a broad analysis, the research aims to clarify the understanding of how carbon is processed and recycled in boreal forest environments. Ultimately, the findings of this research can contribute to a more clarified and deeper view of the carbon cycle and the carbons complicated path.

What happens to the carbon when white rot fungi break down lignin in boreal forest ecosystems? Do the fungi metabolize it intracellularly, or is it converted into

CO_2 and dissolved organic compounds outside of the fungi's metabolic processes? Additionally, is it possible to measure these processes?
Although lignin depolymerization by white rot fungi has been extensively studied (Lundell et al., 2010; Silva et. al., 2021), there is ongoing debate as to whether white rot fungi can utilize lignin as a source of carbon (Cerro et al., 2021).

A smaller experiment to test lignin modification is included in this work, mainly for my understanding and development in this research area.
Lignin consists of two primary types of subunits. One type is derived from coniferyl alcohol monomers, forming guaiacyl (G) units, typically found in softwoods. The other type is formed when the monomer sinapyl alcohol is polymerized, forming syringyl (S) units. Hardwoods often contain a mixed kind of lignin with both G and S units (Novaes et al., 2010).

Coniferyl alcohol plays a significant role in lignin's composition, particularly in the formation of guaiacyl lignin, which is prevalent in softwoods like those found in boreal forests. Understanding the modification of coniferyl alcohol is crucial for explaining lignin degradation pathways, especially in environmental remediation and biofuel production.

In this experiment, we investigated how coniferyl alcohol (see Fig. 1 for molecule) is modified by chemically produced Mn^{3+}.

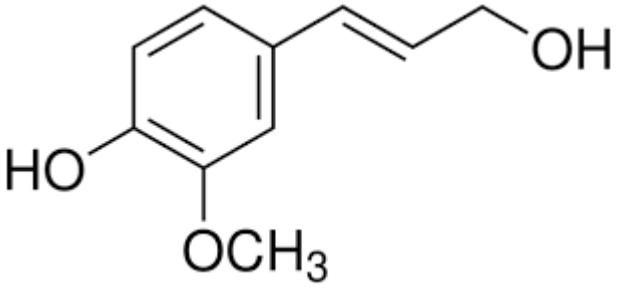

Figure 1. *A coniferyl alcohol molecule*

We compared it to the modification of manganese peroxidase from *Phanerochaete chrysosporium*, a common and well-studied white rot fungi known for its lignin-degrading capabilities (Dashora et al., 2023).

By using Mn^{3+} as a positive control, we can establish a baseline for the oxidation of substrates in the absence of enzymatic activity. This helps distinguish between the direct chemical oxidation by Mn^{3+} and the enzyme-catalyzed oxidation by manganese peroxidase.

The experiment used UV-absorbance to detect any changes in the molecules of coniferyl alcohol. UV-absorbance is a reliable method for detecting changes in the structure of organic molecules, making it suitable for monitoring modifications induced by both chemical and enzymatic reactions. The wavelength appropriate for detecting changes in the coniferyl alcohol molecule is around 280 nm (Lu et al.,

2021). The UV-absorbance spectra of the different treatments were compared to determine any changes in the coniferyl alcohol molecule induced by enzymatic or chemical treatment.

2. Materials and methods

2.1 Literature review

Biology a global approach was used as a base of knowledge about fungi, plant biology, extracellular and intracellular pathways, ecosystems, nutrient cycle and carbon cycle. *Marklära* especially chapter 4 about “soil organic matter and biological processes” was used for basic knowledge of soil organisms.

I did a broad literature search using electronic databases including PubMed, Web of Science, Scispace, and Google Scholar. The search strategy included combinations of keywords such as "white rot fungi", "lignin decomposition", "carbon fate", "saprotrophic fungi", and "boreal forest ecosystems". Filters were applied to limit the search to peer-reviewed articles published in English. Additionally, I manually searched reference lists of the studies and reports I came across. Articles were included if they provided information relevant to the question, but in the beginning, it was hard to determine.

Findings were categorized based on the research in carbon fate, fungi metabolism, extracellular mineralization to CO_2, presence and activity of peroxidase genes, carbon sequestration in the boreal forests, the evolution of lignin decomposition, and if they included methods for studying the path of carbon, for ex. isotope tracing and so on. Which ultimately gave me a broader knowledge base than expected of a narrow question. The different reports were later summarized in methods, results, and valuable insights for my study. Almost every paper contained, for me, new elements of methodology that required more reading.

2.2 An experiment to test lignin modification

The experiment aims to understand differences in lignin modification mechanisms between chemical and biological agents and explore different substrate concentrations to determine the optimal level. The experiment will vary the concentration of coniferyl alcohol to understand its effect on modification by both Mn^{3+} alone and MnP.

Solutions used:

- 50 mM sodium malonate as buffer and stabilizing agent (it forms a complex that maintains Mn^{3+} reactive)
- Evaporated coniferyl alcohol, later suspended in 50mM sodium malonate ($C_3H_3NaO_4$) with pH 4.5.
- 1 mM manganese sulfate ($MnSO_4$) dissolved in 50 mM sodium malonate.
- 0.01 U/ml of Manganese peroxidase from the white rot fungus Phanerochaete Chrysosporium,
- 2.5 mM Hydrogen peroxide (H_2O_2)
- 1 mM Mn^{3+}-ions form of manganese acetate $Mn(CH_3COO)_3$

The different treatments are shown in Table 1. Row A was used as a blank control containing only a buffer. Sodium malonate was chosen as buffer by my supervisor and among the organic acids produced by the fungus in notable quantities, oxalate and malonate are the primary chelators (Wariishi et al. 1992). Chelators like malonate help in releasing Mn^{3+} from the enzyme complex. This means that when MnP oxidized Mn^{2+} to Mn^{3+}, the presence of malonate can assist in efficiently dissociation Mn^{3+} from the enzyme making it more readily available for subsequent reactions (Wariishi et al. 1992).

Row B was used as a substrate control, containing only buffer and coniferyl alcohol. Row C and D served as positive control, containing buffer, coniferyl alcohol, and Mn^{3+} but with two different concentrations, 20 µl with the final concentration of 0,1 mM and 100 µl with the final concentration of 0,5 mM.

Rows E and F contained buffer, coniferyl alcohol, hydrogen peroxide, manganese sulfate, and manganese peroxidase also with two different concentrations. Row G also served as a control, containing buffer, coniferyl alcohol, and hydrogen peroxide. Each well of the microplate contained a total of 200 µl of solution.

Each test was replicated three times with three different concentrations of coniferyl alcohol (0.5 mM, 1.0 mM, and 1.5 mM). We aimed to test the effect of varying substrate concentrations in relation to the MnP and Mn^{3+} to determine the most appropriate levels for optimal activity. In total 61 wells of the microplate 96 were used.

Table 1. *The map of the microplate used in the UV-spectrophotometer when measuring absorbance. All measurements are in µl.*

	Reagents	Coniferyl alcohol [0,5 mM]			Coniferyl alcohol [1,0 mM]			Coniferyl alcohol [1,5 mM]		
A	Buffer	200	200	200	200	200	200	200	200	200
B	Buffer	167	167	167	133	133	133	100	100	100
	Coniferyl alcohol	33	33	33	67	67	67	100	100	100
C	Buffer	147	147	147	113	113	113	80	80	80
	Coniferyl alcohol	33	33	33	67	67	67	100	100	100
	Mn3+	20	20	20	20	20	20	20	20	20
D	Buffer	67	67	67	33	33	33			
	Coniferyl alcohol	33	33	33	67	67	67	100	100	100
	Mn3+	100	100	100	100	100	100	100	100	100
E	Buffer	127	127	127	93	93	93	60	60	60
	Coniferyl alcohol	33	33	33	67	67	67	100	100	100
	$MnSO_4$	20	20	20	20	20	20	20	20	20
	MnP from Fungi	10	10	10	10	10	10	10	10	10
	H_2O_2	10	10	10	10	10	10	10	10	10
F	Buffer	117			83			50		
	Coniferyl alcohol	33	33	33	67	67	67	100	100	100
	$MnSO_4$	20	20	20	20	20	20	20	20	20
	MnP from Fungi	20	20	20	20	20	20	20	20	20
	H_2O_2	10	10	10	10	10	10	10	10	10
G	Buffer	157	157	157	133	133	133	90	90	90
	Coniferyl alcohol	33	33	33	67	67	67	100	100	100
	H_2O_2	10	10	10	10	10	10	10	10	10

3. Literature review

3.1 Boreal forests as an ecosystem

Boreal forests are characterized by cold climates and are particularly populated by coniferous trees, moss, and lichens. The low temperature and acidic soils in boreal forests result in slow decomposition rates, meaning that organic matter accumulates on the forest floor over time (Eriksson et al., 2011; Thirunavukkarasu et al., 2023).

Higher temperatures tend to increase the decomposition rate by boosting the activity of microorganisms. The decomposition process is slower in boreal regions due to the cold climate. Moisture availability also affects decomposition rates. Wetter conditions can enhance microbial activity and decomposition, while dry conditions can slow it down (Thevenot et al., 2010). The acidic nature of boreal forest soils can inhibit decomposition by slowing down enzymes and the activity of decomposing organisms and restricting the types of organisms that can inhabit these environments (Hatakka, 1994). Soil organisms like fungi use oxygen as an electron donor in aerobic respiration, therefore enough oxygen needs to be available in the soil or decomposition will halt. Nutrient-poor soils limit the availability of essential elements for decomposer organisms, which can also slow down decomposition rates. Boreal forests play a crucial role in the global carbon cycle. Because of their slow decomposition rates, boreal forests can store vast amounts of carbon in their vegetation and soils. The amount of organic material in the soil is usually notably larger on average than the amount above ground. However, in a mature spruce forest, there is more carbon in the trees, about 150 tons per hectare, than in the soil, which ranges from 60 to 200 tons per hectare (Eriksson et al., 2011), however, these numbers vary depending on soil quality and latitude. Boreal ecosystems hold approximately one-third of the Earth's carbon stored in land-based ecosystems (Bradshaw & Warkentin, 2015).

The soils found in boreal regions are often acidic and have low nutrient content, which makes them Podzols. The litter found on these soils is also nutrient-poor and has high levels of lignin and/or tannins. It tends to accumulate on the soil surface and decompose without much involvement of the mineral soil. This results in the formation of a humus type called mor, characterized by its morphological features (Eriksson et al., 2011; Thirunavukkarasu, A. et al., 2023). This effect is more pronounced in coarse-textured soils with low nutrient content. In mor, various species of fungi are the dominant decomposers, and within the Basidiomycota phylum, particularly species in Agaricomycetes class (Floudas, 2012).

Nutrient cycling is tightly linked to decomposition processes. Nutrients released through decomposition are taken up by vegetation, and when vegetation dies,

nutrients are returned to the soil, completing the nutrient cycle. Yet, the slow decomposition rates in boreal forests mean that nutrients are often stored for long periods in organic matter, leading to nutrient limitation for plant growth (Eriksson et al., 2011).

During the Calvin cycle, carbon from the atmosphere is absorbed by plants and incorporated into their biomass. As plants grow, some of this biomass ends up in the soil through root production or leaf litter. The organic material in the soil is then broken down by soil heterotrophs, which use the carbon to build their biomass and drive cellular respiration. The process of cellular respiration oxidizes the organic material to carbon dioxide, which is then released back into the atmosphere.

3.2 Organic compounds in plants and litter

Cellulose, hemicellulose, and lignin are three quantitatively important components in plant material. Wood is composed of approximately 25% lignin, 45% cellulose, and 25% hemicelluloses (Novaes et al., 2010). In addition, there are usually smaller quantities of pectin, proteins, lipids, and simple sugars present. However, the proportion of each content varies greatly in different tissues, species, and growth stages (Novaes et al., 2010). Understanding their structural differences and composition is essential for understanding the decomposition and nutrient cycling processes in ecosystems (Hofrichter, 2002).

Cellulose is a long chain of glucose molecules arranged in a polymer (Fig 2) (Martínez et al., 2005). Unlike hemicellulose and lignin, cellulose lacks branching, and its repeated glucose units that provide mechanical strength to plant cell walls (Ahmed et al., 2013; Dashora et al., 2023). Cellulose is the most abundant organic compound and can consist of many (3000-5000) glucose molecules linked together (Lundell, 2010).

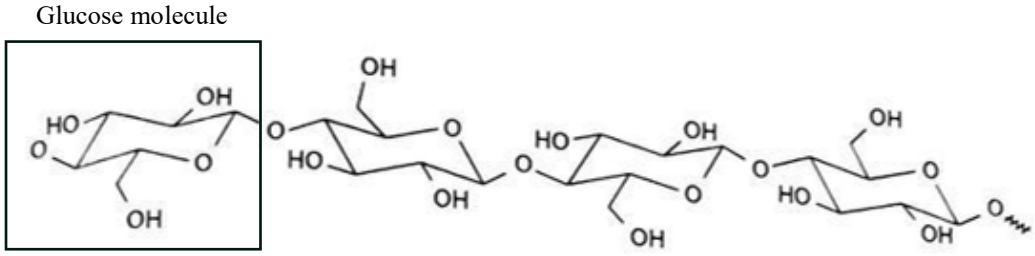

Figure 2. *A cellulose chain with glucose molecules connected with glycosidic bonds. (Eriksson et al. 2011)*

Figure

Hemicellulose is a complex polysaccharide that consists of various monosaccharides like glucose, xylose, mannose, arabinose, and galactose (Fig. 2) (Martínez et al., 2005; Dashora et al., 2023). These monosaccharides are linked by different glycosidic bonds. Unlike cellulose, hemicellulose does not have a systematic repeating structure, it is much shorter and forms amorphous regions within the plant cell wall (Ahmed et al., 2013). It acts as a binding matrix that holds the cellulose fibers together, contributing to the overall structural integrity of the cell wall (Hofrichter, 2002).

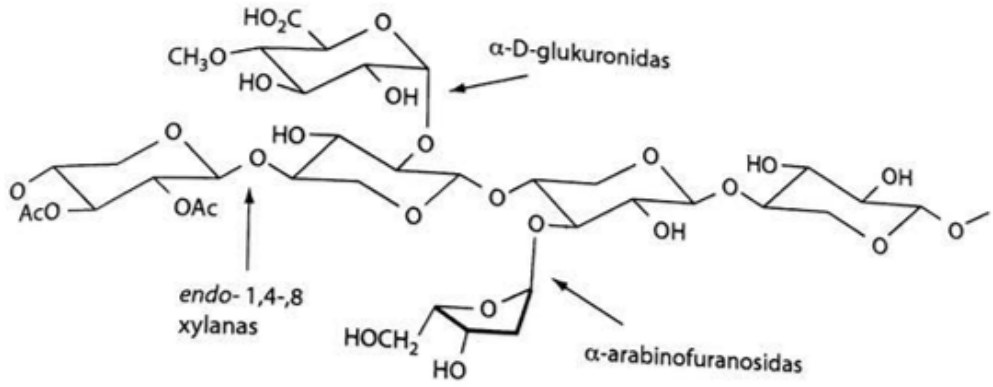

Figure 3. *An example of hemicellulose consisting of many different sugars (Eriksson et al,. 2011).*

Lignin is a complex polymer that gives strength and water resistance to plant cell walls, making them more durable (Lundell, 2010; Hofrichter, 2002).

The presence of lignin makes the plant more resistant to degradation and makes it harder for predators to consume them (Floudas, 2021). Moreover, lignin also plays a crucial role in maintaining the structural integrity of wood, preventing trees from collapsing under their own weight (Novaes et al., 2010; Lundell, 2010).

Lignin is composed of aromatic rings, different alcohols, and various functional groups (Fig. 4) (Floudas, 2021; Hofrichter, 2002; Thevenot et al., 2010). Unlike cellulose and hemicellulose, lignin lacks a defined structure and is highly branched (Martínez et al., 2005; Thevenot et al., 2010). It forms a three-dimensional network that surrounds cellulose and hemicellulose fibers (Novaes et al. 2010; Lundell, 2010; Gonzalez et al., 2020).

Certain aromatic compounds in lignin, such as aromatic hydrocarbons and phenolic compounds, can be toxic or inhibitory to many microorganisms (Undersökningsportalen, 2024).

An aromatic hydrocarbon, also known as an arene, is a type of hydrocarbon that consists of a ring structure with alternating double and single bonds between carbon atoms (Dashora et al., 2023). Lignin contains several types of aromatic

hydrocarbons such as benzene, toluene, and xylene (Hofrichter, 2002; Ahmed et al., 2013). At high concentrations, these compounds can be toxic to some microorganisms (Undersökningsportalen, 2024). Their toxicity stems from their ability to disrupt cell membranes and interfere with cellular processes.

The phenolic compounds such as syringyl, guaiacyl, and p-hydroxyphenyl are released during the process of lignin degradation (Floudas, 2021; Hofrichter, 2002; Ahmed et al., 2013). They are capable of hindering the growth and activities of microorganisms. Furthermore, they can create reactive oxygen species (ROS) within cells (Hofrichter, 2002), resulting in oxidative stress that can damage vital cellular components like DNA, proteins, and lipids (Campbell et al., 2020).

Lignin also contains methoxyphenols which can inhibit microbial growth and activity by disrupting cellular processes and causing membrane damage. Fungi able to degrade lignin are likely to have a very good defense against these types of stress. Moreover, producing reactive oxygen species and other radicals may be part of the fungi's defense mechanism against different microorganisms, which could support the idea that lignin breakdown serves a defensive role by protecting the fungus from microbial competition.

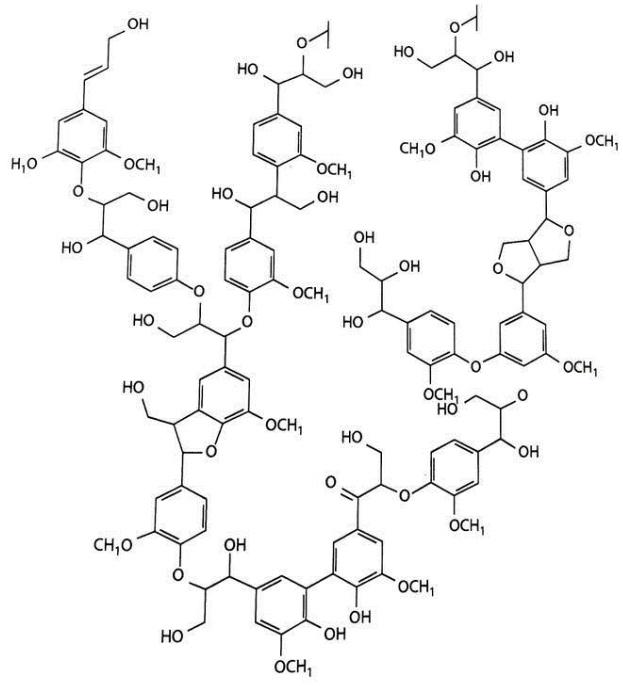

***Figure 4**. An example of a lignin chain containing many different types of aromatic carbons (Eriksson et al. 2011).*

Cellulose and hemicellulose are better sources of carbon when compared to lignin. This is because they are polysaccharides made of simple sugar units and are more abundant in plant biomass. Fungi can easily break down these polysaccharides into simple sugars, which they can utilize more efficiently (Gonzalez et al., 2020).

3.3 Decomposition strategies

3.3.1 Understanding microbial activity and carbon turnover in soil

During the process of microbial turnover, organic carbon is broken down into carbon dioxide through respiration. This process also results in the release of organically bound mineral elements in inorganic form. At the same time, there is an enrichment of persistent (humified) organic material which can potentially be assimilated for the building of new microbial biomass. However, a net increase in microbial biomass only occurs when microorganisms have access to abundant energy, along with available nutrients and favorable environmental conditions (Eriksson et al., 2011).

Soil organisms often experience substrate/energy limitation and temporary abundance when there's an influx of organically bound energy and carbon from plant primary production (Eriksson et al., 2011).

The breakdown of litter increases when there are more easily exchangeable compounds because they provide energy and resources for decomposition. However, this process also depends on the availability of nitrogen and other nutrients, which are necessary for microbial activity and growth. The nitrogen available in the litter gives an idea of the expected turnover rate. The nitrogen content of litter is often expressed as the weight ratio between organic carbon and nitrogen (C/N ratio). This ratio is generally high in the mor horizon (indicating low nitrogen content) and very high in fresh conifer needles (indicating even lower nitrogen content) (Campbell et al., 2020; Eriksson et al., 2011). To clarify, litter with a low C/N ratio tends to decompose faster.

Carbon Use Efficiency (CUE) is a measure of how efficiently organisms, such as fungi and bacteria, convert carbon sources into biomass. In general, approximately 20-40% of the carbon from SOM is used to build biomass, while the remaining 60-80% is released as CO_2 through respiration. When the C/N ratio is over 25, there is an excess of nitrogen, which can lead to an imbalance in nutrient availability. Conversely, a C/N ratio under 25 indicates a lack of nitrogen, which can limit microbial growth since nitrogen is a critical nutrient for building proteins and other cellular components (Campbell et al., 2020; Eriksson et al., 2011). In boreal forests, nitrogen is often the limiting nutrient, making the C/N ratio a crucial parameter for understanding and predicting microbial processes and ecosystem dynamics.

In later stages of litter decomposition, the decomposition rate is inversely proportional to the lignin content, and decomposition may decrease in some cases even at high nitrogen levels (in contrast to the initial decomposition). Forest ecosystems with lignin-rich litter are known to be particularly affected by high nitrogen levels, which can reduce the long-term decomposition rate. Several

hypotheses have been proposed to explain this phenomenon, including the possibility that enzyme activity in lignin-degrading fungi may be inhibited, or that changes in the microbial composition in the soil may occur (Eriksson et al., 2011). The relationships between these factors are not yet fully understood due to several reasons. Soil microbial communities are highly diverse and complex, consisting of numerous species with varying ecological roles. The interactions between different factors are often difficult to isolate as they often interact and influence each other, and the variability of the environment adds further complexity to the relationships.

The activity and growth of soil organisms, particularly microorganisms, largely depend on the chemical composition of the organic material. This activity can be measured by observing respiration rates. When new litter is added to the soil, soluble carbohydrates such as sugar (e.g., sucrose), as well as the most readily exchangeable nitrogen-rich compounds, will be utilized and broken down already during the first days. Somewhat later, proteins and structural carbohydrates such as pectin, hemicellulose, and cellulose are also broken down, while lignin is broken down rather slowly (Eriksson et al., 2011). For the structural carbohydrates, the decomposition process is modified by the fact that these are initially interwoven with lignin to varying degrees. This results in a physical barrier and a delay in the breakdown, as the lignin must be broken down in parallel with the more readily available compounds. The main patterns (soft rot, brown rot, white rot) of wood decay can be identified through macroscopic and microscopic examination in advanced stages (visible decay features, fungal structures, loss of wood characteristics etc.) (Martínez et al., 2005).

The initial turnover is carried out by microorganisms that are specialists in utilizing simple and readily available compounds. These microorganisms have a potentially high growth rate, which contributes to the fact that the rate of turnover of easily metabolized compounds can be very high. In later stages, decomposition is increasingly carried out by slow-growing organisms that are specialized in using more difficult-to-access, structural components (Campbell et al., 2020).

For microorganisms to utilize organic material, it must first be broken down and "mixed" with the microorganisms and this is a common strategy for bacteria. Fungi, on the other hand, grow into the organic material and colonize it. Enzymes play a major role in this process, especially extracellular enzymes, which fungi release outside the cell and are active in the soil solution (Dashora et al., 2023). Once the organic material is accessible, they use additional enzymes to break down the components into smaller parts that they can absorb and use, glucose e.g. (Campbell et al., 2020; Lundell, 2010).

3.3.2 Hydrolytic enzymes

Hydrolytic enzymes are enzymes that act on specific substrates and break chemical bonds with the help of water, for example, C-O and C-N bonds. Because they are

specific in how they cleave, they produce consistent and predictable products (Hofrichter, 2002).

Cellulases are the most fundamental family of enzymes as they cleave the glycosidic bonds in cellulose. This process occurs in three main steps. Endocellulases cleave glycosidic bonds inside the cellulose chain, but they are not as efficient because they release the substrate each time after cutting the chain. When there is a free end of the cellulose chain, exocellulases can take over. Exocellulases, being processive enzymes, can hold onto the substrate and systematically continue to cleave glycosidic bonds along the cellulose chain, releasing cellobiose (a disaccharide). Cellobiose is then cleaved by a β-glucosidase into glucose molecules.

Hemicellulases and cellulases work similarly, but hemicellulases have a more complex role. The hemicellulase family comprises xylanases, mannanases, arabinases, galactanases and xyloglucanases. These enzymes remove side chains and attack the backbone to release oligosaccharides. The oligosaccharides are then degraded into simple sugars (Ahmed et al., 2013).

Because of the cross-linked structure and the heterogeneity of lignin, hydrolytic enzymes are unable to cleave lignin effectively. Consequently, more potent enzymes are required to carry out the cleavage process (Gonzalez et al., 2020).

3.3.3 Oxidative enzymes

Oxidative enzymes are a type of enzymes that aid in oxidation reactions within biological systems. These enzymes help transfer electrons from one molecule to another, usually involving the removal of hydrogen atoms or the transfer of oxygen atoms (Ahmed et al., 2013; Gonzalez et al., 2020).

Lignin Peroxidases (LiPs) are enzymes crucial for the initial attack on lignin. They facilitate the decomposition of lignin by employing hydrogen peroxide to oxidize it, resulting in the formation of free radicals. These agents are powerful oxidizers with high redox potential, capable of breaking down the primary non-phenolic structures of lignin (Ahmed et al., 2013).

Laccases are multi-copper oxidases that catalyze the oxidation of various phenolic and non-phenolic lignin subunits (Ahmed et al., 2013). They use molecular oxygen as an electron acceptor and can oxidize many substrates (Lundell, 2010).

Versatile Peroxidases (VPs) possess characteristics of both LiPs and MnPs, which enable them to break down lignin alone or in conjunction with other enzymes by oxidizing a wide range of lignin-related compounds (Floudas, 2021; Ahmed et. al. 2013).

Manganese Peroxidases (MnPs) also play a significant role in lignin degradation (Hofrichter, 2002). They catalyze the oxidation of lignin using hydrogen peroxide and manganese ions. MnPs are particularly effective in breaking down phenolic

lignin structures (Lundell, 2010), however, these microorganisms cannot break down the tougher non-phenolic lignin (Ahmed et al., 2013). The MnPs are the most common lignin-modifying oxidative enzyme produced by white-rot fungi (Hofrichter, 2002.)

The fungi's hyphae produce hydrogen peroxide and enzymes such as LiPs and MnPs which consist of a heme group that efficiently mediates redox reactions (Ahmed et al., 2013; Martínez et al., 2005).

MnP-reactions for example, start with hydrogen peroxide oxidizing the heme group, which, in turn, oxidizes manganese ions in the soil solution from Mn^{2+} to Mn^{3+} (Ahmed et al., 2013). Mn^{3+} is highly reactive and oxidizes almost anything nearby to regain an electron (Hofrichter, 2002; Lindahl & Tunlid, 2015). This process may demand a lot of energy as the fungus needs to continuously generate hydrogen peroxide to maintain the chemical reaction. However, it can also be powerful because it is nonspecific, unlike the hydrolytic enzymes (Dashora et al., 2023). This aspect can make otherwise inaccessible parts of the organic material available and, thus, profitable (Martínez et al., 2005).

MnP can exist in multiple forms with one fungal strain having up to 11 isoforms (Hofrichter, 2002).

The redox potential of an enzyme refers to its ability to accept or donate electrons during a chemical reaction. In the context of oxidative enzymes like those involved in lignin degradation, having different redox potentials means that they have varying abilities to carry out oxidation reactions (Fig. 5).

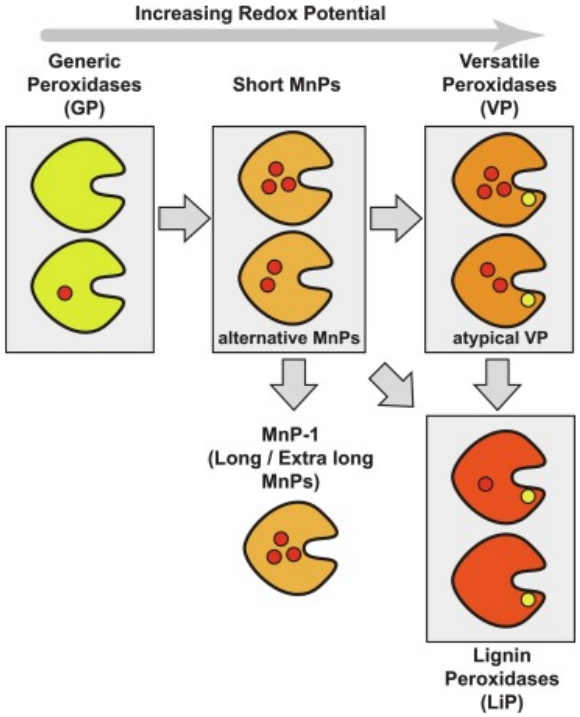

Figure 5. *The oxidative enzymes redox potential (Floudas, 2021)*

Versatile peroxidases have a higher redox potential than generic peroxidases. This increased reactivity allows them to efficiently catalyze oxidation reactions. To clarify, increased redox potential and increased reactivity are related but not the same thing. While a higher redox potential often correlates with increased reactivity in redox reactions, reactivity also depends on other factors like the presence of catalysts, reaction conditions, and the nature of the reactants involved. However, the exploration of the redox potential of versatile peroxidase remains a subject of considerable interest and ongoing research (Dashora et al., 2023).

The differences in enzyme production among various species of white rot fungi have been well-documented. Different species possess distinct sets of enzymes, and this diversity enables efficient degradation of lignin from different sources (Lundell et al., 2010).

3.4 Do white rot fungi utilize carbon derived from lignin?

3.4.1 Understanding the challenges of analysing lignin degradation

The commonly held belief is that white rot fungi break down lignin solely for simpler carbon sources like cellulose and hemicellulose without assimilating the lignin-derived carbon (Hatakka, 1994; Eriksson et al., 2011; Floudas, 2021; Kapich et al., 1999; Gonzalez et al., 2020; Dashora et al., 2023).

Measuring CO_2 production is a common method for assessing decomposition activity, especially in organic matter degradation studies. White rot fungi are aerobic organisms, and the carbon dioxide produced during lignin decomposition originates either from organic compounds being converted to CO_2 extracellularly (with the hydrolytic and oxidative enzymes) or from carbon being metabolized to CO_2 intracellularly. One way to trace the carbon pathway is by using isotopes. Carbon in lignin is labelled with a stable isotope such as ^{13}C or ^{14}C which is unstable, either during the growth of plants (CO_2 in the atmosphere) or through chemical synthesis. This labelling allows researchers to track the labelled carbon throughout the experiment. Lignin labelled with isotopes is exposed to white rot fungi under controlled laboratory conditions. Samples are collected at various time points during the experiment to capture the progression of lignin degradation and the fate of labelled carbon. Samples may include the lignin substrate before degradation, fungal biomass, extracellular products, and CO_2 produced during the experiment. Lignin and its degradation products are extracted from the samples and

are then analyzed to determine the distribution and fate of the labelled carbon. Analytical techniques such as mass spectrometry, gas chromatography (GC) and nuclear magnetic resonance (NMR) spectroscopy are commonly used to characterize the compounds and their isotopic composition (Campbell et al., 2020; Martínez et al., 2005; Shao et al., 2022).

This process may encounter several problems and difficulties, especially in distinguishing between extracellular combustion and intracellular metabolism. There is a risk of labeling other carbon sources present in the growth medium. Cellulose, hemicellulose, and lignin are intertwined components.
Lignin has an unpredictable structure, composed of many different types of phenolic units. It is challenging to determine which specific lignin components are being metabolized and how.

Manganese peroxidases (MnP) can produce CO_2 independent of fungal metabolism (Urziia, 1998). This can complicate CO_2 measurements, making it difficult to differentiate between extracellular conversion and intracellular metabolism. If white rot fungi can take up lignin-derived compounds intracellularly and metabolize them through various pathways, distinguishing between lignin-derived carbon and other carbon sources within the fungal biomass can be complex. Lignin degradation involves a series of metabolic intermediates. Identifying these intermediates and tracing their fate can be challenging, especially distinguishing between extracellular and intracellular pools.

3.4.2 There may be several reasons white rot fungi may not use lignin as a carbon source.

Genetic variation and its impact on lignin degradation efficiency
Even within the same species, genetic variation can affect the efficiency of lignin degradation. Some strains may be more efficient at lignin degradation than others due to differences in their enzyme production or metabolic pathways (Šnajdr et al., 2010; Agosin et al., 1985). White-rot fungi, have large and redundant genomes, especially those capable of lignin degradation. These genomes have multiple genes that encode enzymes with similar functions, which makes it difficult to identify the specific genes responsible for lignin degradation among these redundant gene sets (Dashora et al., 2023). Ligninolytic efficiency varies significantly, but this variability has not been thoroughly documented (Floudas, 2021; Agosin et al., 1985).

Environmental influences on enzyme production and regulation
White rot fungi exhibit a remarkable ability to produce lignin-degrading enzymes, but this production is often regulated by specific environmental conditions or the

availability of substrates. Research suggests that the presence of other carbon sources might influence the production of these enzymes, with some species producing hydrolytic enzymes alongside oxidative enzymes, while others prioritize lignin degradation over cellulose and hemicellulose (Šnajdr et al., 2010; Ahmed et al., 2013; Martínez et al., 2005; Thirunavukkarasu et al., 2023). Despite extensive in vitro studies on the catalytic mechanisms of these enzymes, questions remain regarding how they function together in their natural environment (Martínez et al., 2005). There may be additional mechanisms that have not been discovered yet (Shao et al., 2022). Furthermore, environmental factors such as temperature, pH, and nutrient availability can also influence enzyme production by white rot fungi. In vitro studies have shown that fungi can adjust their enzyme production in response to changes in these factors, as well as in response to different substrate types (Hatakka, 1994).

In natural environments, white rot fungi may interact with other organisms that also utilize lignin or its breakdown products (Šnajdr et al., 2010; Dashora et al., 2023). Each organism must efficiently utilize available resources to survive and grow. White rot fungi, while capable of degrading lignin, may prioritize the utilization of other, more readily available carbon sources like cellulose and hemicellulose. This might help them compete effectively for resources against other organisms. Competition for resources can influence the extent to which white rot fungi utilize lignin as a carbon source.

Challenges and mechanisms in lignin degradation

Lignin is a highly complex polymer composed of various phenolic compounds (Martínez et al., 2005). Even though white rot fungi have enzymes to break down lignin, the process is slow and may be energetically expensive. Fungi may prioritize the use of simpler carbon sources like cellulose and hemicellulose, which are easier to metabolize (Floudas, 2021). Cellulose, hemicellulose, and lignin are three polymers closely bound together and can be densely packed, making it challenging for enzymes or other agents to break them down (because they need to penetrate the substrate first). To comprehend how organisms decompose such a complicated substrate, we need to understand the interaction between different enzymes and biochemical pathways more thoroughly (Lundell et al., 2010; Šnajdr et al., 2010).

Evolutionary adaptations and secondary role of lignin degradation

White rot fungi may degrade lignin alongside other carbon sources like cellulose and hemicellulose (Lindahl & Tunlid, 2015). Lignin degradation may occur as a side activity rather than a primary metabolic pathway (Floudas, 2021; Thevenot et al., 2010). White rot fungi are believed to have evolved the ability to degrade lignin in order to access the cellulose and hemicellulose embedded within the lignin matrix., rather than to use lignin as a primary carbon source. Research indicates that

the enzymatic systems fungi employ to break down lignin are costly in terms of energy and resources. This suggests that the primary evolutionary driver for developing these systems may be to outcompete other organisms for access to the more easily metabolizable carbohydrates, rather than to use lignin itself for growth and energy (Dashora et al., 2023; Silva et. al., 2021).

Nitrogen limitation and energy investment

The decomposition of carbon-rich organic matter with a high C/N ratio is often slow because microorganisms are limited by the availability of nitrogen. Consequently, breaking down lignin may demand a significant energy investment. In response, white rot fungi may prioritize utilizing other carbon sources that offer more energy-efficient pathways for growth and reproduction.

Nevertheless, some basidiomycetes are capable of conquering challenges in wood decomposition, such as the low nitrogen content in wood components (Martínez et al., 2005).

Toxicity in lignin derivates

Breaking down lignin can result in the release of harmful compounds such as phenols and hydrocarbons. To avoid the toxic effects of these byproducts, white rot fungi may limit lignin degradation. Additionally, many species of white rot fungi have developed mechanisms to detoxify these compounds or prevent oxidative stress (Martínez et al., 2005). However, this may also lead to extra energy used.

3.4.3 Lignin as a carbon source?

White rot fungi are traditionally thought to degrade lignin extracellularly, meaning they break down lignin outside their cells and do not use the carbon derived from lignin for their cellular processes. However, recent findings indicate that white rot fungi can utilize lignin carbon intracellularly in vitro, which has significant results for both in vitro (controlled laboratory) and in situ (natural environmental) settings.

If white rot fungi can utilize lignin-derived carbon intracellularly in vitro, it suggests that these fungi have metabolic pathways capable of processing lignin breakdown products within their cells. This contradicts the traditional belief that white rot fungi break down lignin solely outside their cells and suggests there may be significant intracellular enzymatic activity involved.

A 1999 study demonstrated the oxidation of synthetic lignin all the way to CO_2 in a cell-free in vitro system using ^{14}C-labelling. 75% of the carbon was lost as CO_2, while only 6% of the straw was found in the fungus biomass (Hofrichter et al., 1999). The straw contains cellulose, hemicellulose and lignin and was not determined what parts that the fungi metabolized intracellularly. This low CUE suggests that lignin degradation is primarily an extracellular process, where the

fungi secrete enzymes to break down lignin outside their cells (Hofrichter et al., 1999; Lindahl & Tunlid, 2015).

A study from 1985 (Agosin et al. 1985) tested 74 white rot fungi for their ability to break down lignin in wheat straw labeled with ^{14}C. Fifteen strains with high ligninolytic activity were selected for further testing. The fungi showed varied responses in lignin degradation, with some like Pleurotus ostreatus having a high asymptote (77%). The "asymptote" represents the maximum amount of $^{14}CO_2$ released, which indicates the extent to which the lignin has been broken down and metabolized extracellularly by the fungi. The amount of CO_2 produced seems to be directly linked to the amount of lignin decomposed, not considering the possibility of carbon incorporated in fungal biomass.

Another study from 1994 (Hatakka, 1994) demonstrates the effectiveness of white rot fungi in breaking down lignin and the importance of MnP in this process, along with the collaboration of lignin-modifying enzymes. It also provides specific data on the production of CO_2 as a measure of lignin breakdown efficiency and identifies fungi that produce MnP as highly effective at breaking down lignin The study does not explicitly address the fate of the remaining carbon, which raises questions about whether it remains undegraded, is metabolized, and utilized by the fungi, or is stored in other forms. This research gap emphasizes the need for further investigation to ascertain whether the unconverted carbon is stored within the fungi, remains as partially degraded lignin, or is utilized in ways not captured by measuring CO_2.

A more recent study from 2021 (Cerro et al., 2021), used the white rot fungi, T.versicolor and G. subvermispora because of the availability of sequenced genomes and their capacity to break down lignin. In vitro enzyme tests were conducted to test the activity of specific enzymes produced by the fungi that are responsible for breaking down lignin. ^{13}C-isotops in poplar-derived aromatic compounds were used to track the carbon pathway. They determined the level of ^{13}C labeling in intracellular proteinogenic amino acids, which are metabolites derived from central carbon metabolism. Both species exhibited significant enrichment of ^{13}C in intracellular proteinogenic amino acids, confirming that they utilized lignin-derived aromatic compounds as a carbon source. G. subvermispora showed up to 12% ^{13}C labeling, while T. versicolor showed up to 5% labeling, indicating different efficiencies in carbon assimilation from lignin. By analyzing the genomes of these fungi, the researchers hypothesized a complete pathway for the breakdown of 4-HBA which is a lignin unit.

These findings suggest that white rot fungi can break down lignin-derived aromatic compounds within their cells, directing the carbon from lignin into their central carbon metabolism through internal pathways. This discovery could greatly change how we understand carbon cycling in soil ecosystems.

4. Result lignin modification test

UV-absorption spectrum was obtained by scanning the absorbance from 200 to 400 nm on the spectrophotometer at 1 nm intervals. One measurement was taken each day for three days. Row A which served as a blank control showed no sign of contamination or inconsistencies.

The absorbance of wavelengths varies across different concentrations (see Appendix A1-A3 for graphs). This phenomenon can be explained by the Beer-Lambert Law, which asserts that absorbance (A) is directly proportional to the concentration (c) of the substance absorbing light in the solution. The path length (l) that the light traverses and the molar absorptivity (ε) are constants unique to each substance at a specific wavelength, $A = \varepsilon \cdot c \cdot l$.

When the concentration of coniferyl alcohol increases, the absorbance at specific wavelengths increases proportionally. This means that doubling the concentration of coniferyl alcohol will result in doubling the absorbance at each wavelength. This causes the curves to differ in height (absorbance values) but generally maintain the same shape. The curves of the three days of measuring look practically the same meaning the coniferyl alcohol solution is stable over time. There are no significant changes in its concentration, chemical structure, or degradation throughout measurement. This suggests that the coniferyl alcohol does not degrade, react, or evaporate under storage conditions. There were no changes in absorbance in row G containing H_2O_2, no matter the coniferyl alcohol concentrations or depending on time (see Appendix A4-A6).

Mn^{3+} will immediately react with coniferyl alcohol and will only act once; therefore, there should be no changes after day 1. Row C and D showed no difference in absorbance depending on time, therefore only data during the first day is seen in Figure 6 and Figure 7. There is a slight difference in absorbance in well C1-C3, containing 0,5 mM coniferyl alcohol and 0.1 mM Mn^{3+} as seen in Figure 6, and a more extensive difference with 0.5 mM Mn^{3+} concentration as seen in Figure 7. This indicates that something has been somewhat modified, however, the differences in absorbance between row B (only substrate) and rows C and D (Mn^{3+}) are minimal.

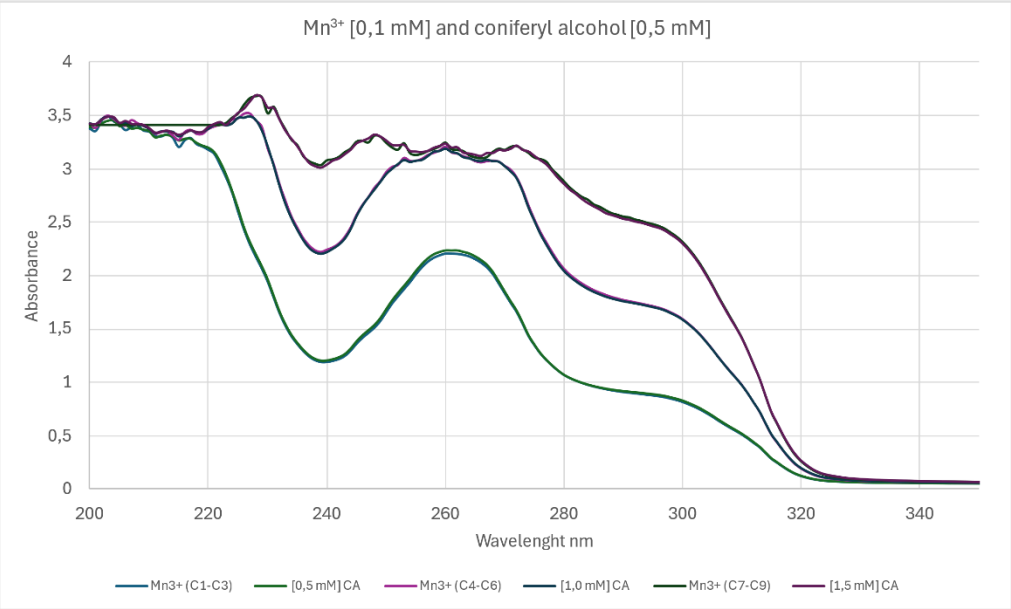

Figure 6. *The average (C1-C9 of day 1) absorbance at different wavelengths of 0.1 mM Mn^{3+} and 0.5-1.5 mM coniferyl alcohol in a buffer was measured. These measurements were compared to the curves in Appendix A1-A3.*

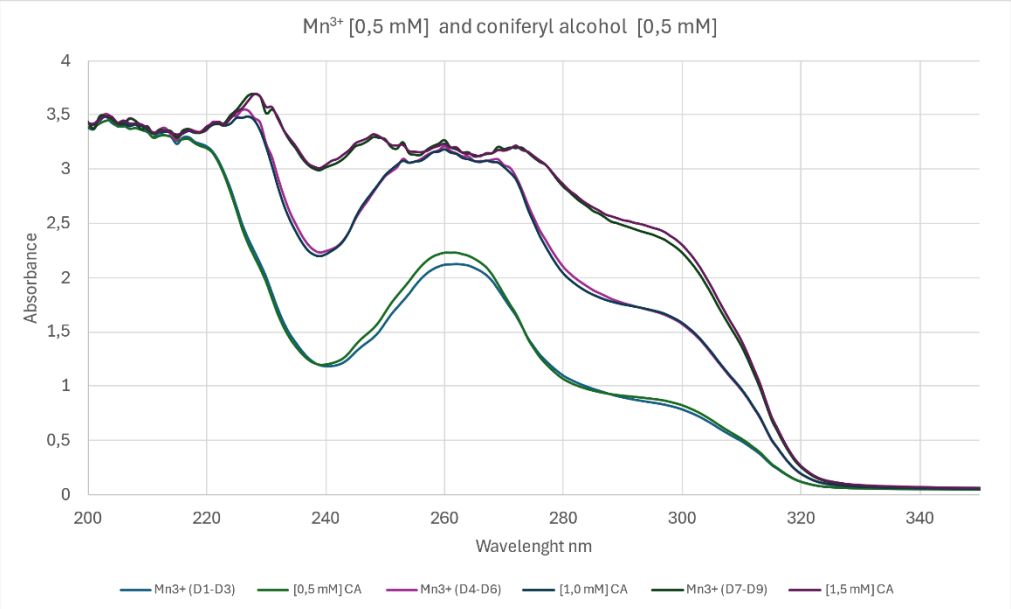

Figure 7. *The average (D1-D9 of day 1) absorbance at different wavelengths of 0.5 mM Mn^{3+} and 0.5-1.5 mM coniferyl alcohol in a buffer was measured. These measurements were compared to the curves in Appendix A1-A3.*

The wells containing MnP, H_2O_2 and Mn^{3+}, rows E and F showed a bigger difference in absorbance depending on time, therefore all three days are seen in Figures 8-13. The most extensive difference in absorbance is seen on days 2 and 3, especially in Figures 8, 11, and 12. In both Figures 8 and 11 0,5 mM coniferyl alcohol was used, and in Figures 11 and 12 the double amount of MnP was used.

The difference between the reactions with Mn^{3+} alone and the MnP is that Mn^{3+} can be produced continuously if the enzyme is active and there is still H_2O_2.

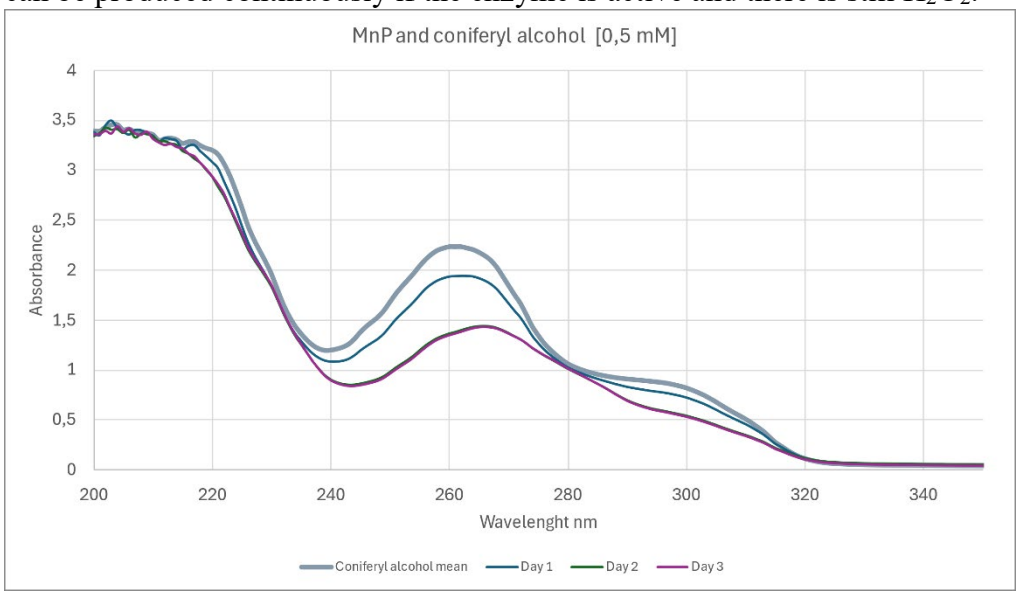

Figure 8. *Measurements were done for three days, and the average (E1-E3) absorbance at different wavelengths of 0.0001 units of MnP and 0.5 mM coniferyl alcohol in a buffer was recorded. These measurements were compared to the substrate curve in Appendix A1.*

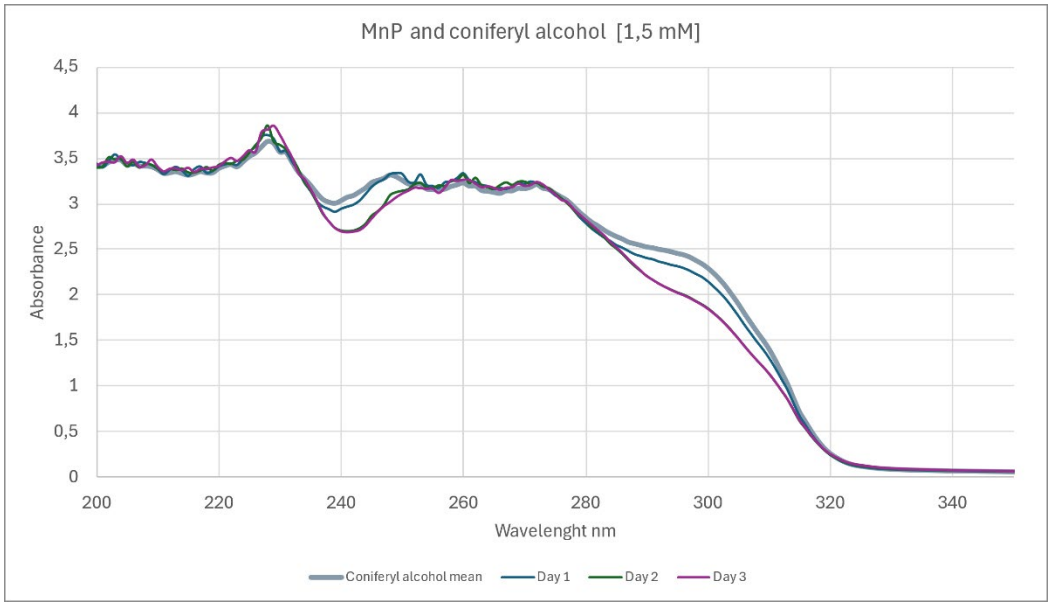

Figure 9*. Measurements were done for three days, and the average (E4-E6) absorbance at different wavelengths of 0.0001 units of MnP and 1.0 mM coniferyl alcohol in a buffer was recorded. These measurements were compared to the substrate curve in Appendix A2.*

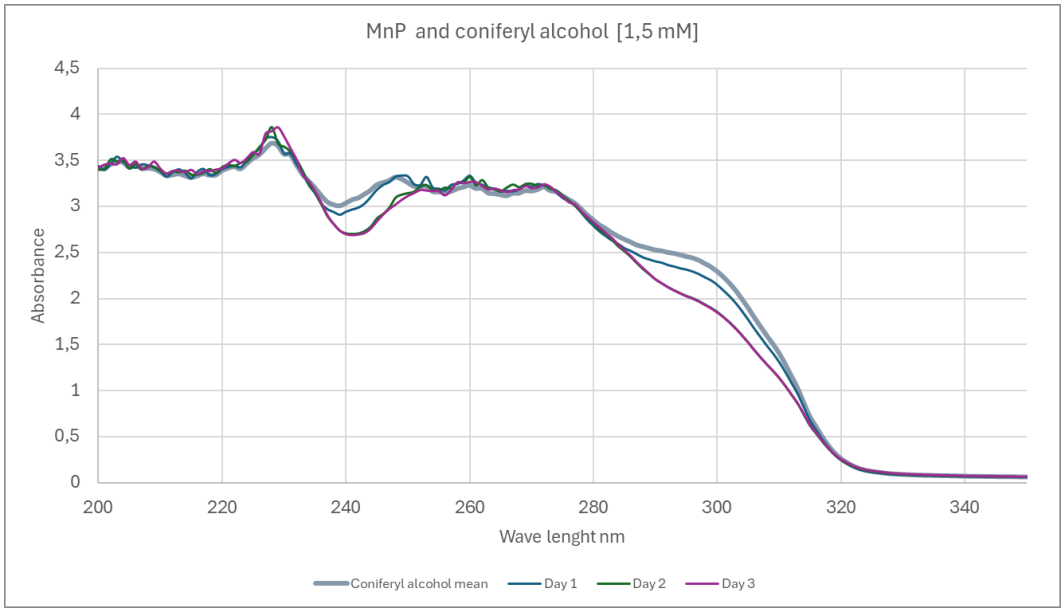

Figure 10. *Measurements were done for three days, and the average (E7-E9) absorbance at different wavelengths of 0.0001 units of MnP and 1.5 mM coniferyl alcohol in a buffer was recorded. These measurements were compared to the substrate curve in Appendix A3.*

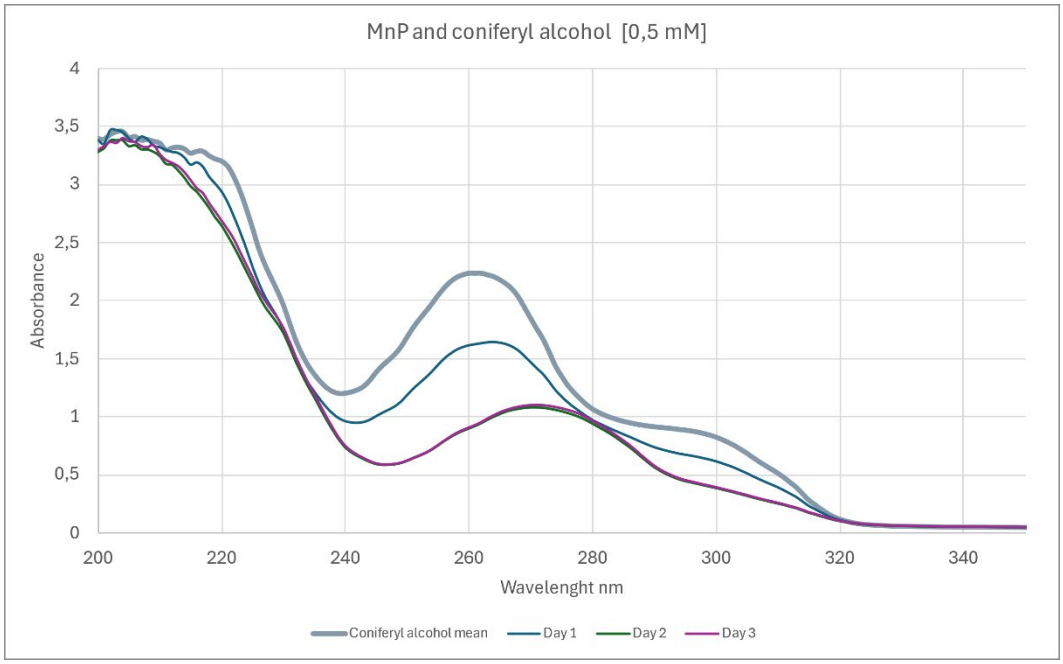

Figure 11. *Measurements were done for three days, and the average (F1-F3) absorbance at different wavelengths of 0.0002 units of MnP and 1.5 mM coniferyl alcohol in a buffer was recorded. These measurements were compared to the curve in Appendix A1.*

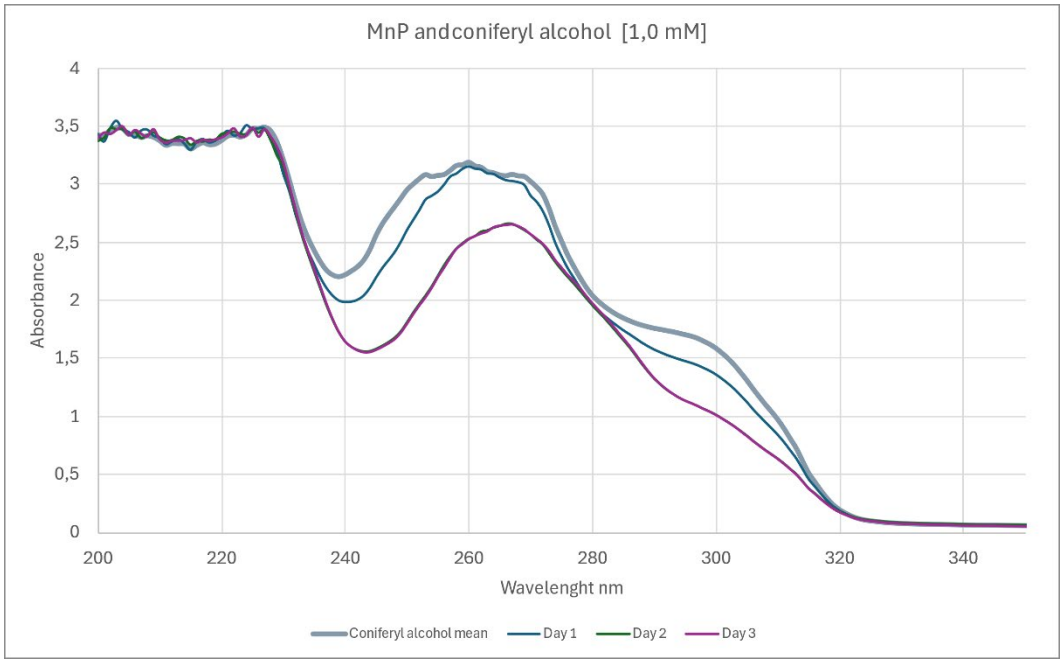

Figure 12. *Measurements were done for three days, and the average (F4-F6) absorbance at different wavelengths of 0.0002 units of MnP and 1.5 mM coniferyl alcohol in a buffer was recorded. These measurements were compared to the curve in Appendix A2.*

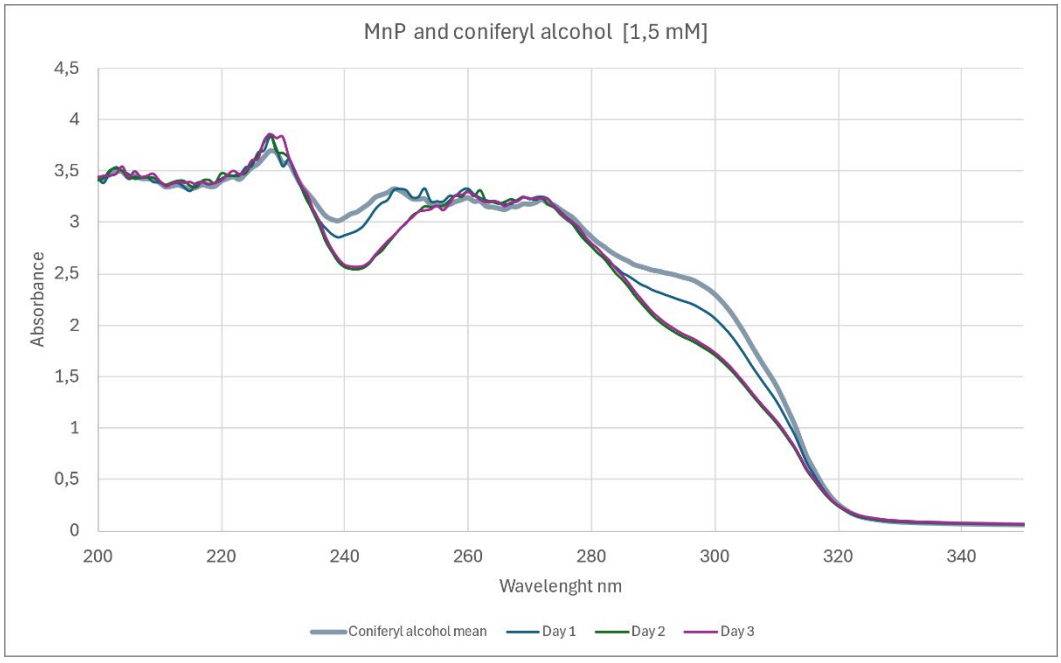

Figure 13. *Measurements were done for three days, and the average (F7-F9) absorbance at different wavelengths of 0.0002 units of MnP and 1.5 mM coniferyl alcohol in a buffer was recorded. These measurements were compared to the curve in Appendix A3.*

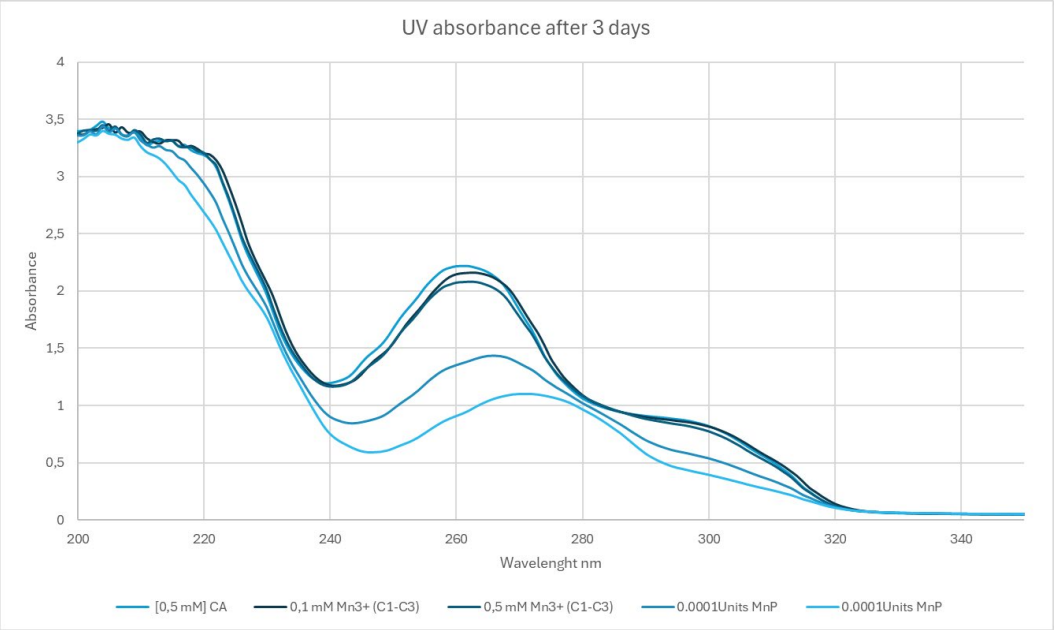

Figure 14. *The different reactants MnP and Mn^{3+} in relation to the substrate coniferyl alcohol at 0.5 mM concentration.*

The most appropriate level of concentration of coniferyl alcohol for optimal activity seems to be at 0.5 mM. This is shown in all figures (6-14).

For future experiments, a higher concentration of Mn^{3+} should be considered or, maybe other stabilizing agents (e.g., oxalic acid) should be included (Yaver et al., 2003). To figure out how much Mn^{3+} is needed to achieve the same result as manganese peroxidase is recycling, we first need to understand how the enzyme works and the chemistry of the reaction. Based on the premises that the enzyme is still active and the available hydrogen peroxide, a hypothesis can be calculated.

For example, to determine how many times 0.0002 units of MnP can recycle 20 µl f 1 mM Mn^{2+} into Mn^{3+}, given 10 microliters of 2.5 mM H_2O_2, we must start with the enzymatic activity of MnP.

According to the webpage of the distributor Sigma-Aldrich where the MnP was obtained, 1 unit of MnP oxidizes 1.0 µmole of Mn^{2+} to Mn^{3+}/minute at pH 4.5 and 25°C (Sigma-Aldrich, n.d.) and the ratio between $Mn3^{+}$ and H_2O_2 is 2:1 (Wariishi et al. 1992). Table 2 shows a calculated hypothetical regeneration of Mn^{2+} by MnP.

Table 2. *Calculation of a hypothetical regeneration of Mn^{2+}*

Calculations of H_2O_2 in µmole	
Volume of H_2O_2	10 µL = 0.00001 L
Concentration of H_2O_2	2.5 mM = 2.5×10^{-3} M
Moles of H_2O_2 (m=c×v)	2.5×10^{-3} M ×0.00001 L =2.5×10^{-8} moles = **0.025 µmoles**
Stoichiometry of Mn^{3+} and H_2O_2	
Reaction ratio	2 $Mn3^{+}$: 1 H_2O_2
0.025 µmoles H_2O_2 will produce **0.005 µmoles Mn^{3+}**	
Oxidation Capacity of MnP	
1 unit of MnP oxidizes 1.0 µmole Mn^{2+}/min to Mn^{3+}	0.0002 units of MnP will oxidize **0.0002 µmoles** of Mn^{2+}/min to Mn^{3+}
Calculation of regeneration	
Number of regenerations	$\frac{0.05\ \mu moles\ Mn^{3+}}{0.0002\ \mu moles/minute} = 250$
To achieve the same oxidation result as 0.0002 units of MnP, which can regenerate 250 times, we need a total of 0.05 µmoles of Mn^{3+}	

However, in row C, 20 µl of a 1 mM Mn^{3+}, which corresponds to 0.02 µmoles Mn^{3+} was used. In row D, 100 µl of a 1 mM Mn^{3+}, which corresponds to 0.1 µmoles Mn^{3+} was used. Row C only contained nearly half of the amount of Mn^{3+} and Row D contain the double amount. These wells of the microplate didn't yield the results compared to the ones with MnP.

5. Discussion

5.1 Literature review

Boreal forests, like all ecosystems are complex and dynamic environments, which makes it difficult to isolate and control variables that affect carbon fate from lignin decomposition. The fate of carbon can vary in different locations and periods, making it challenging to generalize results across biomes. White rot fungi interact with diverse microbial communities in the soil, including other fungi, bacteria, and archaea, which can influence the fate of carbon and complicate the interpretation of results (Šnajdr et al., 2010). Studying these microbial communities offers a more comprehensive understanding of lignin degradation processes. In these environments, various microorganisms collaborate by secreting a range of biocatalysts that work synergistically. While such cooperation is common in nature, culturing all the necessary organisms in the laboratory can be challenging. Laboratory experiments may not fully replicate natural ecosystem conditions and may lead to inconsistencies between laboratory and field results (Lundell et al., 2010; Shao et al., 2022; Šnajdr et al., 2010).

Therefore, the methods used to measure carbon fate, such as isotope labeling and microbial community analysis (respiration, degradation), have limitations in accuracy, sensitivity, and specificity (Agosin et al., 1985; Hatakka, 1994). Obtaining representative samples from boreal forest ecosystems without altering them may be challenging. There is a lack of direct evidence to conclusively determine whether white rot fungi utilize lignin as a carbon source or if it is mineralized extracellularly. Many studies rely on indirect measurements and assumptions. Several papers state that "White-rot fungi are the only known organisms that can completely break down lignin to carbon dioxide and water" (Hatakka, 1994; Eriksson et al., 2011; Floudas, 2021; Kapich et al., 1999; Gonzalez et al., 2020; Dashora et al., 2023), but there are uncertainties if they use the lignin-derived carbon for their metabolism and growth.

We need to dig deeper into how fungi metabolize lignin-derived carbon inside their cells. Understanding these processes is key to seeing how carbon cycles through forest ecosystems. If fungi can not only break down lignin but also use its carbon for growth, it could greatly affect soil carbon dynamics and shift their

ecological roles. This highlights the need for advanced genetic tools and protocols to study these interactions in natural settings.

5.2 Lignin modification test

Conducting “simple” absorbance experiments can be an eye-opening experience for understanding the complexity of more advanced experiments. It was very rewarding to experiment with the help and support of my supervisor. Working with a spectrophotometer provides hands-on experience with common laboratory equipment. I was allowed to learn how to formulate a hypothesis, design experiments to test them, and analyze the results critically. Understanding the importance of controlling variables (e.g., concentration, pH, temperature) is essential for obtaining reliable data. But the most important thing for me was to connect theory to practice.

The differences seen in Figure 6 and 7 are small. Still, they are larger when the concentration is higher. This indicates that the reaction is too weak and that the ratio of Mn^{3+} might be too low compared to the substrate. The calculation made in Table 2, compared to the amount of Mn^{3+} used in Figure 6 and 7 shows that something did not go as planned. Mn^{3+} without MnP may not be as stable or as effective in the reaction conditions compared to the enzymatic action of MnP. Free Mn^{3+} might be prone to side reactions or maybe a different concentration of the stabilizing agent should be considered, there may not be enough complexing ligands available when the reaction happens at once, compared to when MnP is being able to recycle Mn^{2+} to Mn^{3+}. Additional research needs to be done to draw a conclusive result.

6. Conclusion

Recent studies have altered our understanding of white rot fungi. Traditionally, it is believed that these fungi only break down lignin outside their cells and don't use carbon derived from lignin for their own cellular processes.

However, new evidence suggests that white rot fungi can process lignin carbon inside their cells, revealing potential metabolic pathways for handling lignin breakdown products within fungal cells. This new perspective opens up exciting possibilities for understanding how these fungi contribute to carbon cycling in forest ecosystems.

Still, the research is conducted in vitro and only 12 % respectively 5 % of lignin-derived carbon was found in fungal biomass. It is rather new research, and it was also the only study found that suggests that white rot fungi can utilize carbon derived from lignin. More research needs to be done to draw a conclusive conclusion.

The reviewed studies highlight the complexity and variability in how of white rot fungi degrade lignin. Some fungi are particularly efficient at breaking down lignin, thanks to their ability to produce a variety of enzymes, such as manganese peroxidase (MnP). Despite the extensive research done on lignin degradation, there is still no conclusive answer on what happens to the carbon. This unanswered question points to a gap in our knowledge.

Recognizing that white rot fungi can use lignin-derived carbon inside their cells has implications for both ecological research and biotechnological applications. It suggests these fungi might play a more significant role in carbon cycling than we previously thought, affecting soil carbon dynamics, and possibly aiding in carbon sequestration. To fully understand these processes, we need advanced genetic tools and protocols to study these fungi in their natural environments. This deeper understanding could reveal much more about the ecological roles and capabilities of white rot fungi.

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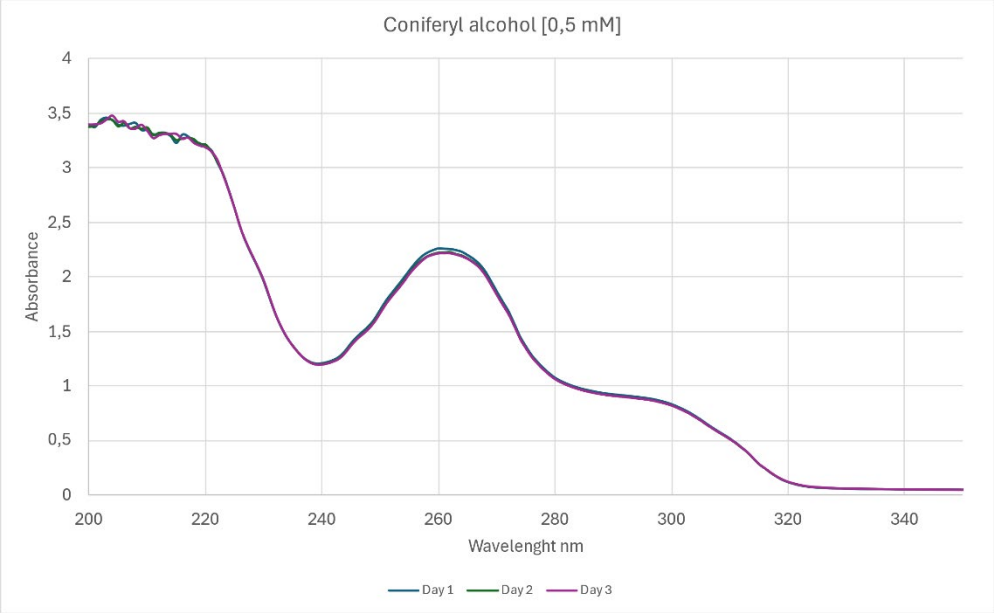
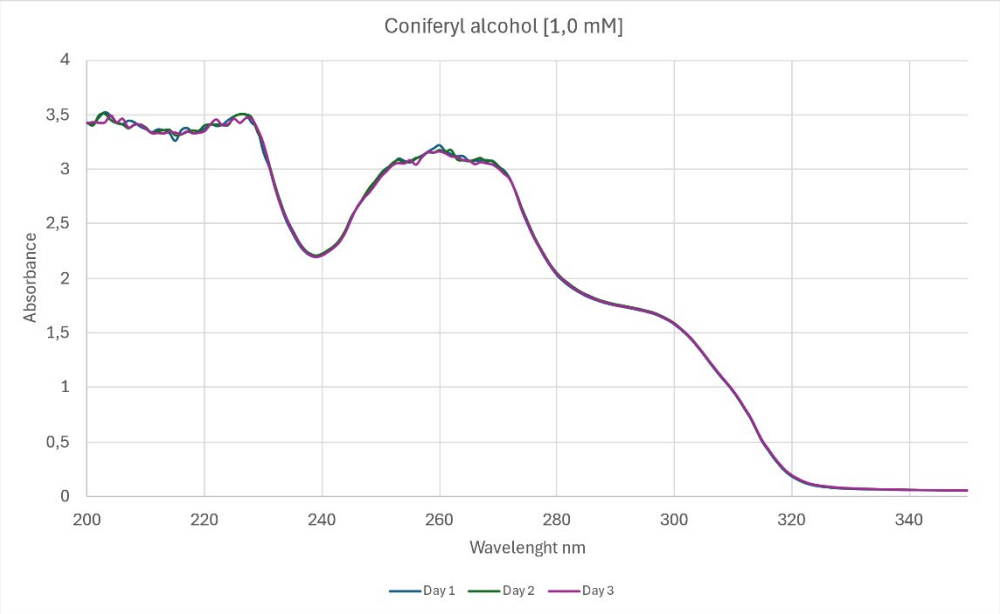
Acknowledgements

I want to thank my supervisor Erica Packard for encouraging and helping me and for being very enthusiastic about my project. And I’m also very grateful for my friends and my partner for being sounding boards and helping me when I got stuck in the writing process.

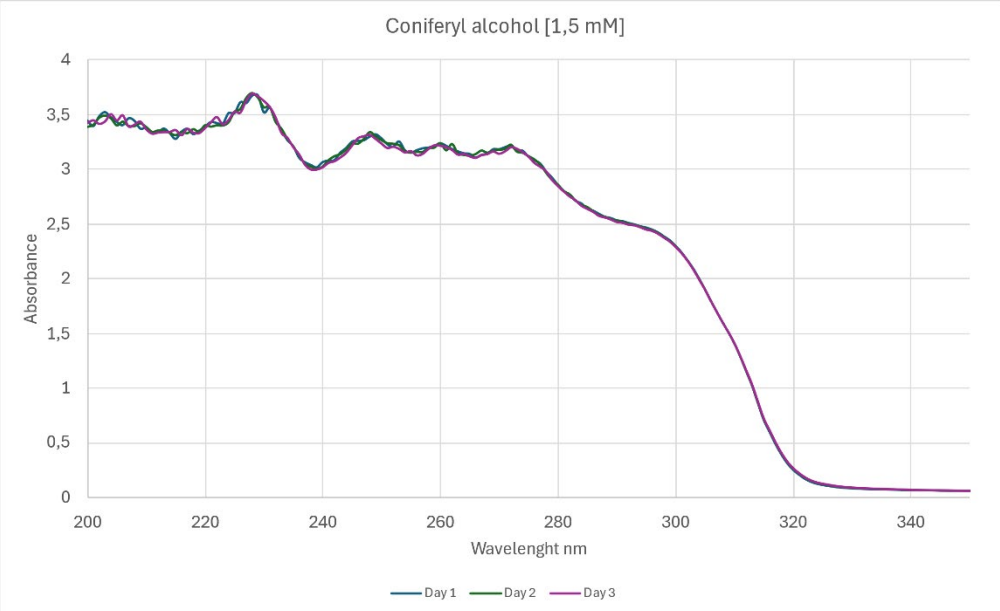
Appendix A



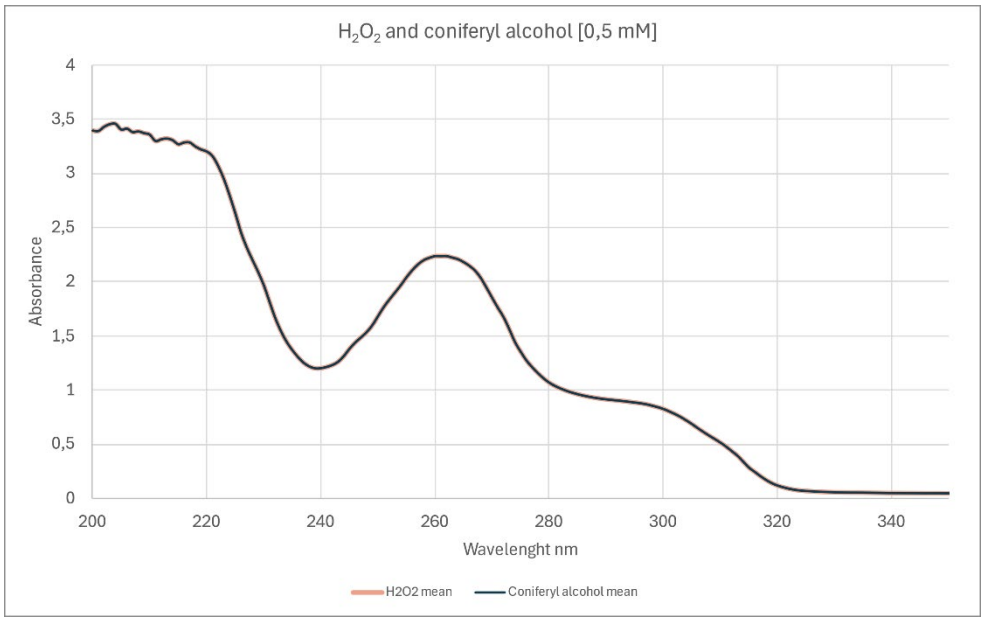
Appendix A1. *The average (B1-B3) absorbance at different wavelengths of coniferyl alcohol at 0.5 mM concentration in a buffer.*



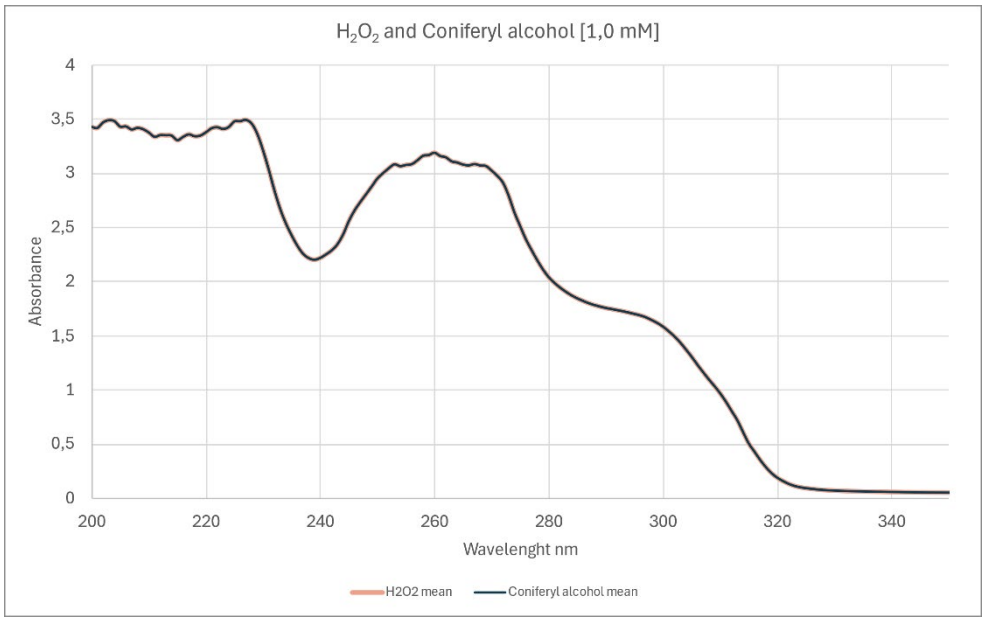
***Appendix A1**. The average (B4-B6) absorbance at different wavelengths of coniferyl alcohol at 1.0 mM concentration in a buffer.*



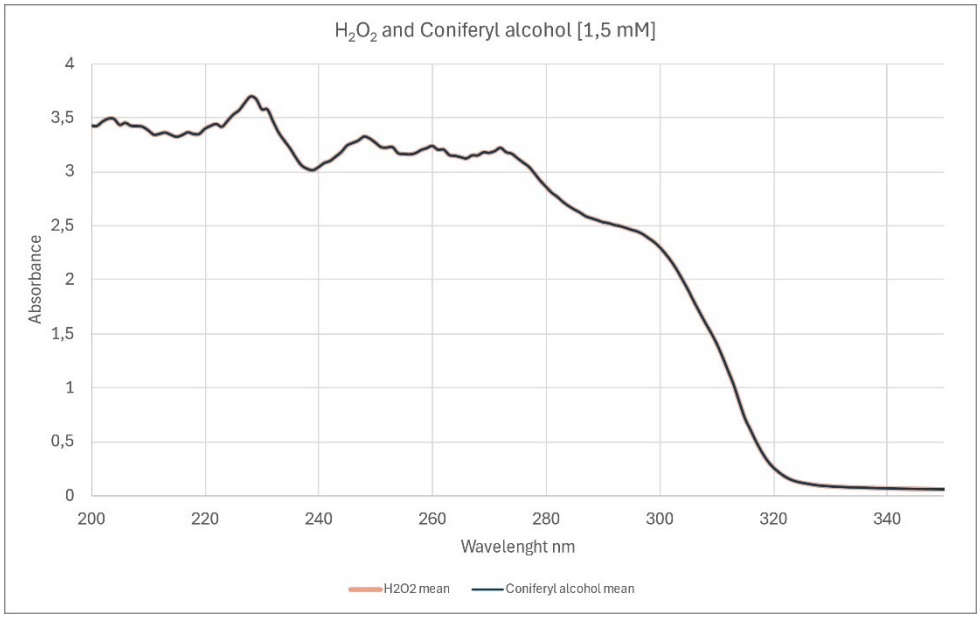
***Appendix A2**. The average (B6-B9) absorbance at different wavelengths of coniferyl alcohol at 1.5 mM concentration in a buffer.*



Appendix A4. *The average (G1-G3) absorbance at different wavelengths of H_2O_2 and coniferyl alcohol at 0.5 mM concentration in a buffer.*



Appendix A3. *The average (G4-G6) absorbance at different wavelengths of H_2O_2 and coniferyl alcohol at 1.0 mM concentration in a buffer.*



Appendix A4. *The average (G7-G9) absorbance at different wavelengths of H_2O_2 and coniferyl alcohol at 1.5 mM concentration in a buffer.*

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