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–
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Study programme: Master of Science in Ecology and Environmental Management
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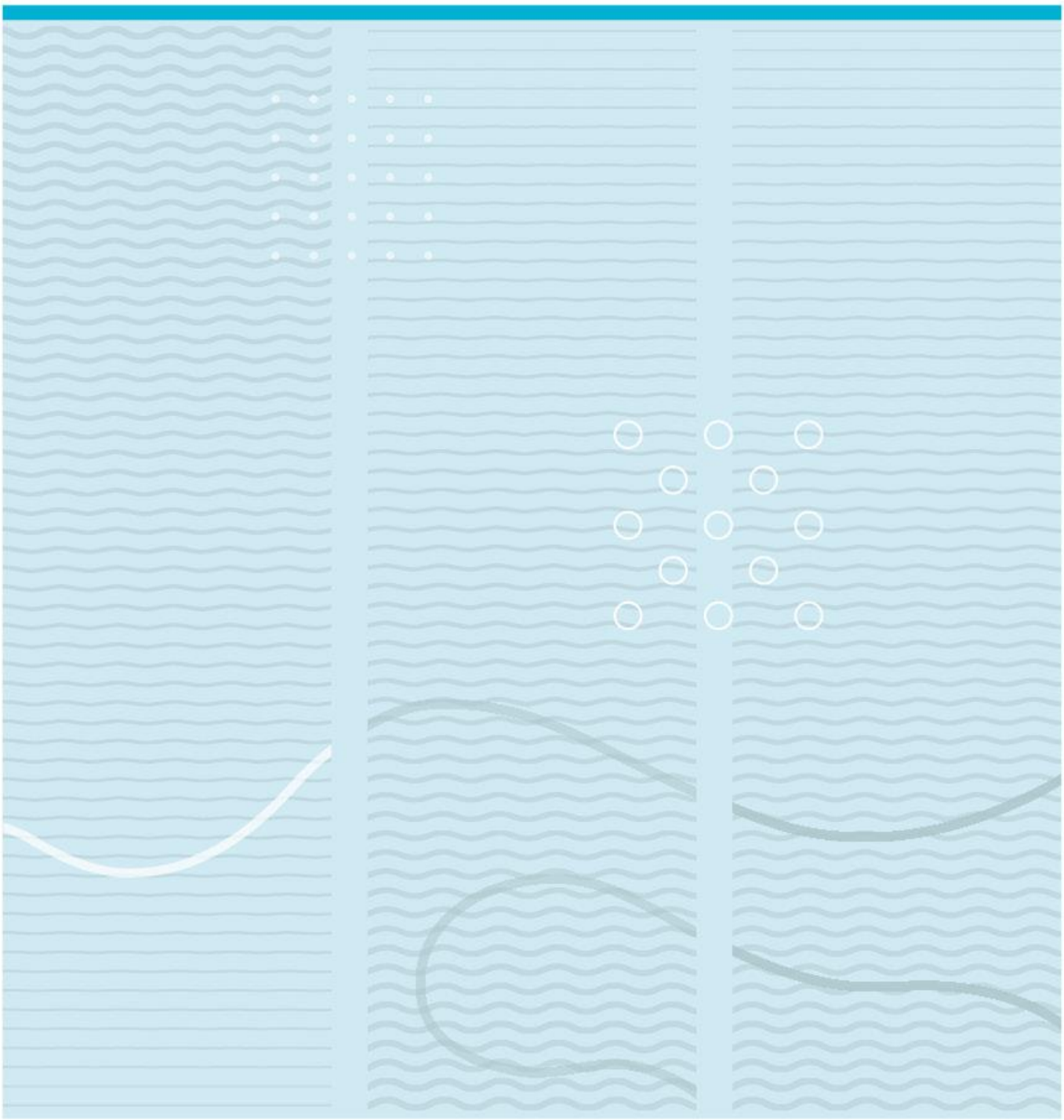
Fungal succession in a cultured Norway spruce (*Picea abies*) forest.



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Preface

This is the final master's thesis conducted at the University of South-Eastern Norway by faculty of mathematics, natural sciences and environmental sciences in study program Master of Science in ecology and environmental management. The master's thesis was written over two semesters, autumn 2022 and spring 2023.

An important starting point for this master's thesis was the growing research interest related to genetic analysis of environmental DNA. The greatest inspiration for me was the study of fungal communities, which allowed me to conduct a wide range of research in the direction in which I wanted to develop.

I would like to express my deepest appreciation to Jørn Henrik Sønstebø for his time, knowledge, invaluable substantive support, and his boundless commitment. I would also like to thank PhD student Sofie Geck for the friendly atmosphere and valuable tips she gave me during the laboratory work. I would like to express my special thanks to my Jo-Tellef and my parents Elzbieta and Janusz for the great support, patience and understanding they showed me during the writing of my master thesis.

Bø i Telemark 15.mai 2023

Abstract

One of the most central topics in ecology is the relation between biodiversity and ecosystem. Many wood-inhabiting fungi are decomposers, with an important role in the circulation of matter and creation of habitats for other wood decay fungi in forest ecosystems. However, little is known about the relation between succession of fungal community and different stages of decomposition, especially in the early stage before the establishment of fruiting bodies. In addition, little is known also about the establishment of rare polypore fungi that are used in monitoring old growth forests.

I studied fungal communities in (i) Standing trees with a hint of dryness, (ii) Laying trees that are partially decomposed, have still bark and are exposed to bark beetles and (iii) laying trees that has no bark and is highly decomposed, in an old-cultivated forest in Norway. Based on eight million reads of the dsDNA ITS region, 1 275 501 OTUs were detected in 48 DNA samples. The great richness of fungi was presented by 58 % were Ascomycota, 35% were Basidiomycota, 1 % were Mortierellmycota and 1% were Mucoromycota. In total 88 orders were recovered and the most common of these were Helotiales (39.2%), Polyporales (14%), Saccharomycetes (13.3%). A total of 31 polypores (order; Polyporales and Hymenochaetales) were identified.

In the first place, the fungal species richness was relatively similar among stages “Laying partially decomposed” and “Laying highly decomposed”. There were nonsignificant differences, nevertheless Shannon index was highest during the early stage. The fungal species richness decreased with wood decay which is of great importance for fungal community. Beta diversity displayed highly similarity among stages “Laying highly decomposed” and “Laying partially decomposed”. The fungal species composition changes during substrate succession. Interestingly, the high values in shift community in stage “Laying highly decomposed” indicated strongly dominated fungal microbiome with yeast fungi (Saccharomycetes).

Secondly, the trophic mode analysis assigned the fungal OTUs to the main trophic modes: animal pathogen, ectomycorrhizal, endophyte and saprotrophs. Saprotrophic fungi increased in proportion when the wood is more decayed.

Finally, the rare species of polypore fungi used in monitoring of old-cultured forests are more common at later stages but can be established early. Species such as *F.rosea* and *P.centrifuga* were registered in all stages of decomposition.

List of abbreviations used

bc	Barcode
Bp	Base pair (nucleotide)
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleaside triphosphate
dsDNA	Double stranded deoxyribonucleic acid
fiTS7	Forward primer
ITS4R	Internal transcribed spacer (Reverse primer)
Kb	Kilo-bases
Lat	Latitude
Long	Longitude
M	Chromatography-purified DNA fragments with reference bands
mL	Milliliter
mm	Millimeter
ng	Nanogram
PCR	Polymerase chain reaction
pM	Picomolar
qPCR	quantitative/ Real-time polymerase chain reaction
U	Units
µl	Microliter
Fmol	Femtomole

Keywords:

Decomposition stages
DNA metabarcoding
Fungal diversity
Next-generation sequencing
Wood-inhabiting fungi

1.0 Introduction

1.1 The first establishment phase of wood-inhabiting fungi

Many of wood-inhabiting fungi are decomposers, with an important role in the circulation of matter and creation of habitats for other wood decay fungi in forest ecosystems (Boody, Frankland, & Van West, 2007, ss. 171-172). Wood-inhabiting fungi can grow inside trees for many years, in a way that is invisible to humans because they do not produce fruiting bodies (Abrego, 2022). Fungal wood decay weakens the wood mechanically, even in the initial phase, before we can see the signs on the tree. Fruiting bodies occurring on a tree is usually a sign of an advanced decomposition stage (Schwarze, 2004). Thus, while the absence of fruiting bodies does not guarantee the absence of decomposition, the presence of fruiting bodies indicates its advanced stage (Tattar, 1989).

The ecology of wood-inhabiting fungi has been investigated for more than a century, mainly focusing on identification and conservation of declining species (Baldrian, 2016). As a result, many studies on wood-inhabiting fungal communities have been conducted on old, decayed logs or focused on investigating diversity linked to the decomposition process on the stage of the life cycle when the fungi are visible as fruitbodies.

DNA metabarcoding can identify fungi at all parts of the life cycle. It involves the parallel sequencing of a DNA region (DNA barcode) from all fungi in an environmental sample and successive identification through comparison with sequence databases. The advantage of this method is the possibility of determining organisms also in developmental stages difficult to identify by traditional methods (Lamb, et al., 2018). Metabarcoding of forest trees is extremely useful in identifying fungal species, often in the absence of ephemeral, short-lived fruiting bodies. Study can take place in different years and seasons (Tedersoo, et al., 2022). Based on the fruit body morphology and anatomical structure of the fruiting body, the species can be identified with observation and knowledge by analysis of the Internal Transcribed Spacer (ITS) region of environmental DNA.

1.2 Trees indicator of species diversity

A unit of dead wood includes tens to hundreds of fungal species with different ecological functions that vary throughout the life cycle, such as wood decomposing (saprotrophic fungi), mycorrhizal, lichenized, parasitic and endophytic fungi (Ottosson, et al., 2015); (Koskinen, et al., 2022, ss. 13-14). Wood decaying fungi are classified as soft rot, white rot

and brown rot (Srivastava, Kumar, & Singh, 2014), according to the type of decay that they cause: soft rot enzymatically breaks down cellulose in woody tissues, white rot can degrade lignin and brown rot can degrade hemicellulose and cellulose - the main components of wood, as a result of which wood changes its properties. This process provides them with the energy and nutrients necessary for life, while at the same time leading to the breakdown of the wood (Nguyen , Wikee, & Lumyong, 2018, ss. 1075-1076).

Several researchers point out that a high diversity of wood-dwelling species is correlated with the amount of dead wood in a forest. Many rare species of wood decay fungi are almost exclusively found in old-growth forests that often contain dead wood of different size and age (Brandrud, Skarpaas, & Svergrup-Thygeson, 2013, s. 10). However, in many cultured forests (where forestry has affected history relatively recently) we often find large and decomposed trees that could be a good habitat for many rare fungi, nevertheless there are often very few rare fruiting bodies found. For example, in my study area, which has a history of intensive selective logging, there are a relatively large amount of dead wood, but few rare species have been identified. One reason for the lack of fruit-bodies from rare species can be that they are unable to establish themselves due to out-competition, wrong environmental conditions or because it is a long time until they make fruit bodies that we have not seen yet. Much of the previous research on tree-dwelling fungal communities points out that trees often form fruiting bodies when there is no food and different strategies (Rigling, 2021). Some form fruiting bodies straight away while some form fruiting bodies after 40-50 years when the tree has been decomposed (Rigling, 2021, s. 30). This is interesting for estimating population size.

1.3 Aims

The aim of the study is first to study the succession of the fungal community from live standing trees to dead well decomposed trees. I expect that the trophic mode of the fungi will vary at det different decay stages, and the hypothesis is that saprotrophs will increase in proportion when the wood is more decayed.

Secondly, I study the timing of establishment of rare polypore fungi that are used in monitoring of old growth forests. I hypothesis that many of the species that are rare in fruitbody surveys can establish early and can be found in all stages of decay.

2.0 Materials and methods

2.1 Study area

The study area is a spruce dominated old cultural forest located in Villmyrene in Bø in Midt-Telemark municipality in Vestfold and Telemark County (see Appendix 1). The terrain is flat at approximately one hundred meters above sea level. In the last 100-150 years, this forest has probably formed after a period of grazing. Today the area is a forest dominated by spruce and pine with some birch in between. The forest area is surrounded by cultivated soil (see Figure 1). The bedrock in the study area consists of granitic gneiss, it is a hard and very low-lime rock that provides poor vegetation (NGU, Nasjonal berggrunnsdatabase, 2022). The surface organic layer consists of peat (NGU, Nasjonal løsmassedatabase, 2022).

Figure 1. The current section map marked with white line shows Villmyrene, Bø i Telemark. The section marked with green polygon (5325,395m^2) shows sampling collection area (QGIS, 2022).

2.2 Sample collection

The sampling was performed on September 14, 2022. Field work included observation and selection of twelve spruce trees that were almost the same size. I sampled trees in three distinct categories with varying decay stage (i) Standing trees with a hint of dryness, (ii) Laying trees that are partially decomposed, have still bark and are exposed to bark beetles and (iii) laying trees that has no bark and is highly decomposed (Table 1, Figure 2).

Sawdust samples were taken separately from the basal with the electric drill. Four samples have been taken per tree with intervals of approximately one meter. The drill bits were adapted to the quality of trees. The 16-millimeter drill bits were used on dead wood, because the laying wood was decomposed and led to it being easier to drill and collect sawdust. The 10-milimeter drill bit has been used on more compact trees. Between each sample, the drill has been sterilized with a liquid of 50% chlorine and 50% distilled water. Sawdust from individual holes were collected in 50-millimeter tubes and stored at -20 °C until DNA extraction.

Table 1. Dataset from field work, registered spruce trees with UTM-coordinates

Extract	*Category*	*Lat*	*Long*	*Drill mm*
1	Standing hint of dryness	59,413223	8,973721	10
2	Standing hint of dryness	59,413225	8,973498	10
3	Standing hint of dryness	59,413088	8,973773	10
4	Standing hint of dryness	59,413089	8,973239	10
5	Laying partially decomposed	59,413127	8,973855	16
6	Laying partially decomposed	59,413163	8,973852	16
7	Laying partially decomposed	59,413037	8,973883	16
8	Laying highly decomposed	59,413194	8,974269	16
9	Laying partially decomposed	59,413176	8,974294	16
10	Laying highly decomposed	59,413160	8,973952	10
11	Laying highly decomposed	59,413283	8,973957	10
12	Laying highly decomposed	59,413348	8,974324	10

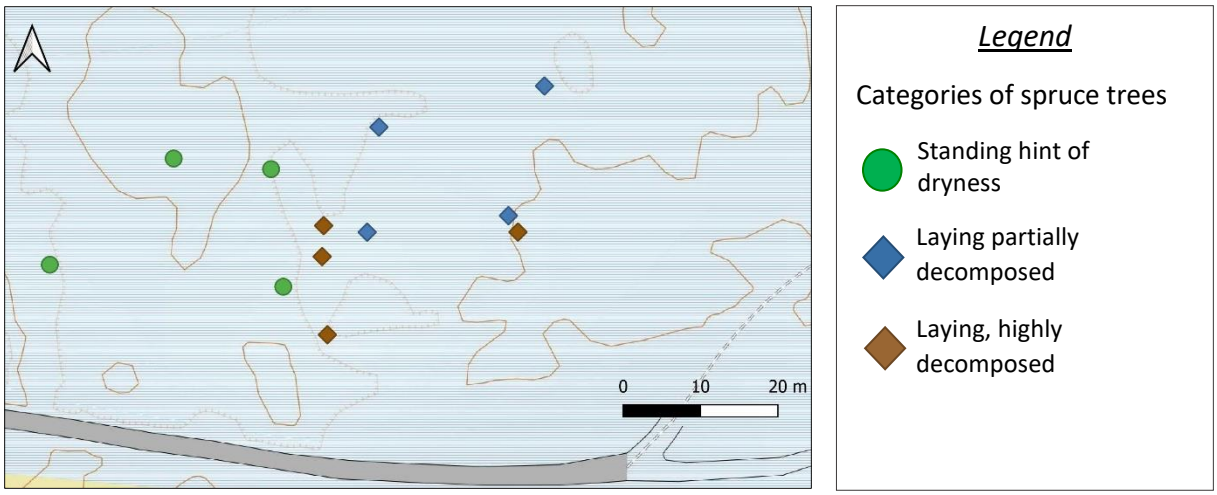


Figure 2. Registred spruce trees in Villmyrene in Bø, Midt-Telemark (QGIS, 2022).

2.3 DNA extraction

I isolated the genomic DNA of the sawdust using the DNeasy PowerSoil ® Pro Kit Handbook by QIAGEN, following the protocol for DNA extraction, described in the instruction manual (Qiagen, 2021). I followed the procedure with some simple modification. Before starting, frozen samples were left to thaw at room temperature for a few minutes.

For each of the 48 samples 150 mg of sawdust was added to PowerBead Tube using a pre-sterilized spatula. The PowerBead tube already contained beads to assist in homogenizing the mixture and protect DNA from degradation. Each sample was mixed with 800 µl of CD1 solution. For those samples that was taken from trees characterized by dryness (see Table 1) an extra 200 µl of solution CD1 was added. DNA was eluted by filtering the supernatant with a 100 µl of solution C6. I also included a negative control for DNA extraction. The resulting concentration of DNA was measured by using the NanoDrop Lite ND-1000 spectrophotometer. Eluted DNAs were stored at -20°C until the next procedure.

2.4 Polymerase Chain Reaction (PCR)

The fungal ITS2 was amplified using the primers fITS7 (Ihrmark, et al., 2012) and a modified ITS4 (White, Bruns, Lee, & Taylor, 1990). Both forward and revers primers were tagged with barcodes to allow multiplexing of samples during sequencing (see appendix 2 for tag and primer sequence). I performed two alternative amplifications using either KAPA Taq polymerase or Phusion polymerase.

The KAPA PCR was done in a 0.2 mL PCR-tubes in a total volume of 25 µl including 12.5 µl of Kapa taq Extra hotstart readymix 2X, 1.5 µl primer mix (10 µl of each primer) and approximately 15ng of DNA. The PCR was run on BioRad iCycler 582BR thermal cycler with the following conditions: After 1-min initial denaturation at 95°C, the reaction mixture was run through 35 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 55°C and elongation for 1 minute at 72°C. The final elongation runs through 1 cycle for 7 minutes at 72°C. Followed by an incubation at 4°C in refrigerator. Some of sample concentrations were too low to measure, those samples were rerun with diluted DNA (5 µl DNA extraction, 20 µl nuclease-free water). I followed the procedure using the same protocol for Kapa Taq polymerase.

Phusion PCR was done in a 0.2 mL PCR-tubes in a total volume of 25.5 µl for each PCR reaction. Master mix including 12.5 µl of Phusion™ High-Fidelity DNA Polymerase, 1.5 µl nuclease-free water, 5 µl of GC enhancer, 1.5 µl primer mix and 5 µl of DNA. The PCR was run on a BioRad iCycler 582BR thermal cycler with the following conditions: thermal cycle stated with 30-sec initial denaturation at 98°C for one cycle. 35 cycles of denaturation at 98°C for 10 sec, annealing on 98°C for 10 sec, extension at 72°C for 15 sec. The final extension was run for one cycle at 72°C for 5-min and at the end incubation at 4°C in refrigerator. I also included a negative control for the PCR reaction.

2.5 Quantity and quality control of PCR

The quality of PCR product was assessed with 1% agarose gel electrophoresis using E-Gel^{R}Technical Guide (Invitrogen, 2008). Ten samples were checked for the DNA analysis in E-Gel EX system and run time was set to ten minutes. E-gel^{TM} Power Snap Camera was used to visualize the fragments as tapes on the gel with UV light. Resulting amplification patterns are presented in appendix 3.

The quantity of the DNA was measured using Invitrogen Qubit Fluorometer with dsDNA quantitation and high sensitivity assay kit. The quantities were used to pool approximately equal amount of DNA from the tagged amplicons.

2.6 DNA-library

The DNA-library were constructed by the Ion Plus Fragment Library kit (ThermoFisher scientific, 2016). DNA library and sequencing was done twice because the result of the first run gave insufficient number of reads (approximately two million reads). Both runs were done using the same kit.

For both runs, I prepared diluted mixture for pool and end repair based on 2.1 µl of pooled amplicon (48 ng/ µl) and 76.9 µl nuclease-free water to obtain suitable the appropriate total volume of 79 µl. For the first run, the non-barcoded library was prepared with 2 µl of adapters and 2 µl of dNTP mix. For the second run, the barcoded library was prepared with 2 µl Ion P1 adapter and 2 µl of Ion Xpress™ Barcode 10. The adaptors ligation was run on a BioRad iCycler 582BR thermal cycler with the following conditions: Start temperature at 25 °C for 15 minutes. Hold temperature at 72 °C for 5 minutes. Fallowed by incubation at 4°C in refrigerator for up to 1 hour. For both runs the adapter-ligated and nick repaired DNA was purified with AMpure™ EP Reagent suitable for 400-base-read. Eluted DNAs were stored at -20°C until the next procedure.

2.7 Quantitative polymerase chain reaction (qPCR)

To identify the quantity of the library and the library dilution factor I performed a qPCR reaction using the Ion Library TaqMan™ Quantitation Kit (ThermoFisher scientific, 2022) on a StepOnePlus™ Real-Time PCR thermocycler system, following manufacturer's instructions.

For both runs the control library included 425 µl of master mix based on 280 µl of taq polymerase, 280 µl of assay, 280 µl of nuclease-free water and 5 µl of eluted DNA. For the first run the master mix was distributed equally in a 96-well PCR plate. The first series with 1:20 dilution and second series with 1:800 dilution. For the second run the master mix was distributed equally in a 48-well PCR plate. The first series with 1:800 dilution and second series with 1:20.000 dilution. Both runs included negative control based on <5 µl master mix and 5 µl nuclease-free water. The thermal cycling condition was compatible with >300-bp libraries followed by UDH incubation at 50 ℃ for 2 min, polymerase activation at 95 ℃ for 2 min and 40 cycles at 95 ℃ for 15 sec and at 60 ℃ for 1 min.

2.8 Library sequence

The libraries were diluted to approximately 60pM and were prepared for sequencing on an Ion Chef (Thermo Fisher) following the Ion 510™ & Ion 520™ & Ion 530™ Kit protocol. Sequencing was done on an Ion 530™ chip in a S5 DNA sequencer (Thermo Fisher). The resulting sequences were downloaded from the Torrent server as fastq files. The reads were demultiplexed and filtered using cutadapt v. 4.1 (Cutadapt, 2023). Further filtering, identifying amplified sequence variants (ASVs) and taxonomic identification were done using the dada2 pipeline (Callahan, McMurdie, & Holmes, 2023).

2.9 Statistical methods

Since my aim was to investigate the succession of the fungal community from live standing trees to almost dead trees and study how trees at different decomposition stages can be used as indicator of species diversity, using metabarcoding. I only selected those fungal species that were detected DNA-based data.

Statistical analyses of the microbial community compositions were performed using R version 4.1.3 via the platform RStudio 1.10.7 (2022.12.0) (see appendix 4 for data analysis pipeline). The packages 'phyloseq' (McMurdie & Holmes, 2013) and 'tidyverse' (Wickerham, Averick, Bryan, & Chang, 2019) was pre-loaded. 'phyloseq' was chosen specifically for handling and analyzing high-throughput microbial community data. 'tidyverse' is a set of compatible packages with powerful tools for data manipulation and visualization. Samples with less than 5000 reads were removed from the further analyses. The statistical analysis was based with and without rarefication of the reads. Since the dada2 pipeline often identifies several ASVs to the same species I used the tax_glom function in phyloseq to merge these ASVs before downstream analyses.

2.10 Alpha diversity analysis

The Shannon-Wiener diversity index (Konopinski, 2020) was used as a measure of alpha diversity in all three different stages. In addition, observed species richness was calculated. Prior to analysis, the metadata had to be created, metadata included seven variables that provided information about samples (see appendix 5). Taking into account the estimates within samples like diversity by calculating a wide selection of metrics using the read abundance of each species in each sample. Further, I used the Wilcoxon test (Yinglin, 2020) to verify if the mean values of different stages differ significantly from each other.

2.11 Beta diversity analysis

Beta diversity refers to the distribution of species in the communities, i.e., the similarity of the samples in terms of species composition. I chose principal-coordinate analysis (PCoA) scatterplot based on Bray Curtis distance (Dill-McFarland, 2017) measure to visualize the beta diversity. The species table was thinned to a uniform subcollection depth, i.e., the minimum number of sequences to avoid bias in the estimates considering that there was a significant range in the number of sequencings across the samples.

2.12 Functional guild analysis

Using database FUNGuild (Nguyen, et al., 2016) I compared the fungal functions and identified the ecological guild to taxonomically parse fungal species. FUNGuildR (RDRR.io, 2021) was used to assign trait information to the species identified in my samples, using the FUNGuild database. The frequency of the assigned ecological guilds in the different decomposition stages were visualized using pie charts in ggplot2.

3.0 Result

3.1 DNA extraction and PCR reaction

Of a total of 48 samples 29 samples were successfully amplified and achieved the correct concentration in relation to the extraction result in the first PCR. With dilution of DNA from each extraction technique, successful PCR amplification was achieved with 9 of 19 remaining samples. The remaining 10 samples were successfully amplified with new primers sets using Phusion polymerase (see appendix 6 for result of DNA extraction and PCR reaction).

3.2 Taxonomic results

In total, about eight million reads resulted from the sequencing and after demultiplexing in cutadapt and filtering for length and quality in dada2, 1 275 501 reads were left for downstream analyses. The final number of reads varied from about 500 to 120 000 among the registered 42 samples, with a mean value of 31011.15 (see

Figure *3*). Samples that are missing in figure 4 are samples with barcode 6, 8, 54, 16 in stage "Standing hint of dryness", 23, 58, 59 in stage "Laying partially decomposed" and 37 in stage "Laying highly decomposed".

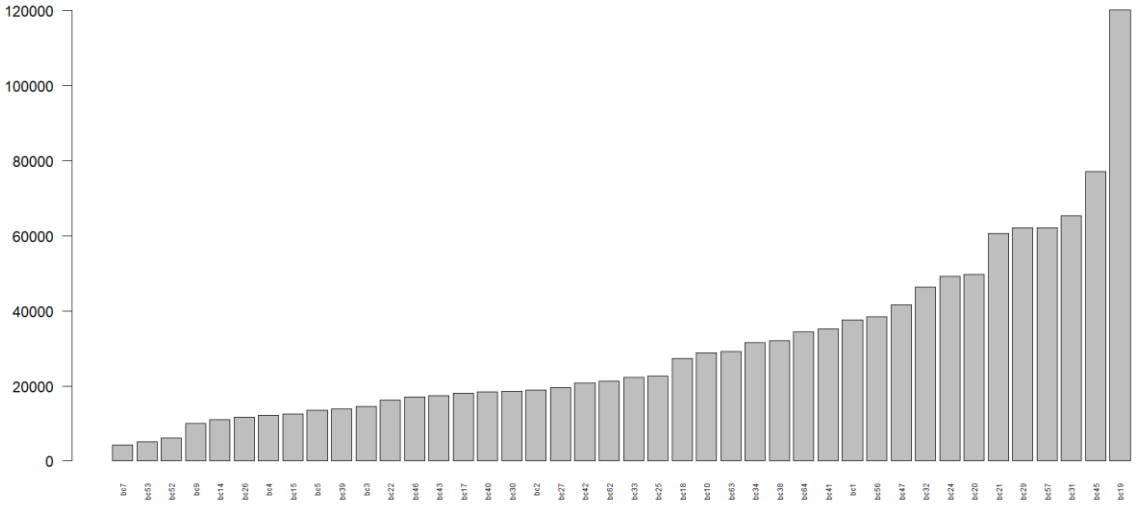


Figure 3. A barplot showing variation in numbers of sequence reads generated for sawdust samples.

Of the fungal OTUs recovered 58 % were Ascomycota, 35% were Basidiomycota, 1 % were Mortierellmycota and 1% were Mucoromycota. I also got a few reads from plants, but those were too few to interfere with the analysis. In total 88 orders were recovered and the most

common of these were Helotiales (39.2%), Polyporales (14%), Saccharomycetales (13.3%). The most common classes were Agaricomycetes (18.2%), Leotiomycetes (15.1%) and Sordariomycetes (12.8%). Those classes were distinguished by a slightly larger number in laying trees.

A total of 31 polypores (order; Polyporales and Hymenochaetales) were identified by DNA in study area, but five of those are undefined species. Species that appeared in all three different stages of decomposition were *Fomitopsis pinicola, Antrodia serialis, Mycoacia fuscoatra, Hyphodontia pallidula, Resinicium bicolor, Tubulicrinis borealis* and *Trichaptum abietinum*. Species that appeared only in stage "Standing hint of dryness" were *Ceriporiopsis portcrosensis* and *Schizopora paradoxa*. Species that appeared only in stage "Laying partially decomposed" were Cr*ustoderma dryinum, Dacryobolus sudans, Postia definitum, Ceriporiopsis cyanescens, amyloporia sinuosa, postia undosa, Phellopilus nigrolimitatus* and *Xylodon rimosissimus*. The species *Steccherinum fimbriatum* appeared only in stage "Laying highly decomposed". The number of abundances varies depending on the stage, but no species is abundant enough to dominate the substrate (see Figure 4 and appendix 6).

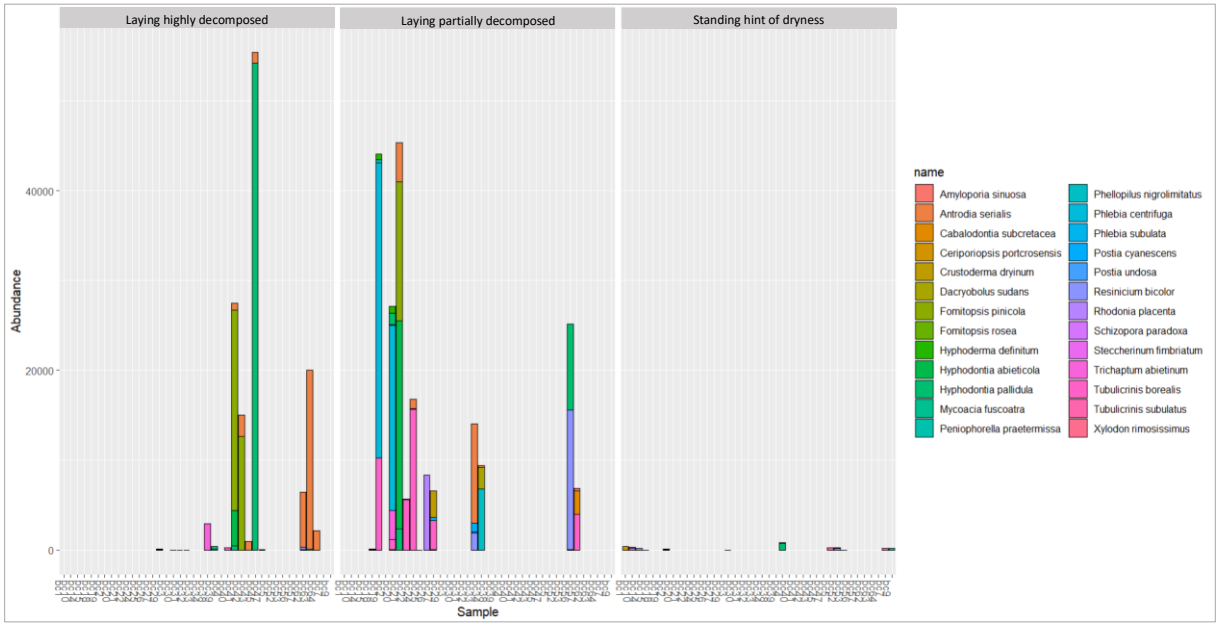


Figure 4. Bar chart showing the distribution of the polypores (order; Polyporales and Hymenochaetales). Bar composition in term of three decomposition stages; Standing hint of dryness, Laying highly decomposed, Laying partially decomposed. Each bar represents one barcode.

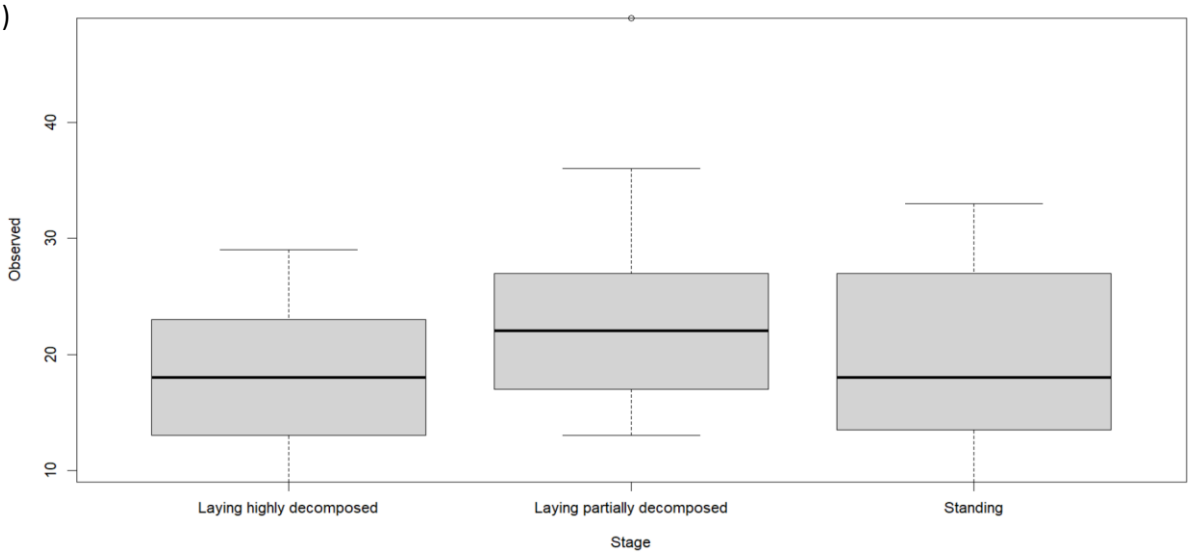
3.3 Alpha diversity

Figure 5 shows the diversity, as measured by the observed species richness and the Shannon index, within samples at the different stages of decomposition. In the observed species richness (Figure 6a) the stage “Laying partially decomposed” (with a mean value of 24.07692) has a slightly higher diversity than the stage “Laying highly decomposed” (with a mean value of 18.26667) and stage “Standing hint of dryness” (with a mean value of 19.83333). Between stages “Laying partially decomposed” and “Laying highly decomposed” were the p-value 0.1063. Between stages “Laying partially decomposed” and “Standing hint of dryness” were the p-value 0.2879. This means that the differences are not statistically significant.

In the Shannon index (Figure 6b) the stage “Standing hint of dryness” (with a mean value of 2.280017) has a higher value than stage “Laying partially decomposed” (with a mean value of 1.627774) and “Laying highly decomposed” (with a mean value of 1.251035). Between stages “Laying partially decomposed” and “Laying highly decomposed” were the p-value 0.02872. Between stages “Laying partially decomposed” and “Standing hint of dryness” were the p-value 0.001553. This means that the differences are statistically significant.

In addition, compared between lower observed species richness and the greater Shannon index in stage “Standing hint of dryness” may be because there are many observed species that are equally common. Stages “Laying partially decomposed” and “Laying highly decomposed” have a higher observed species richness than Shannon index, this may have a connection with certain species being very dominant. These species are often rare or have few sequences.

(a)



(b)

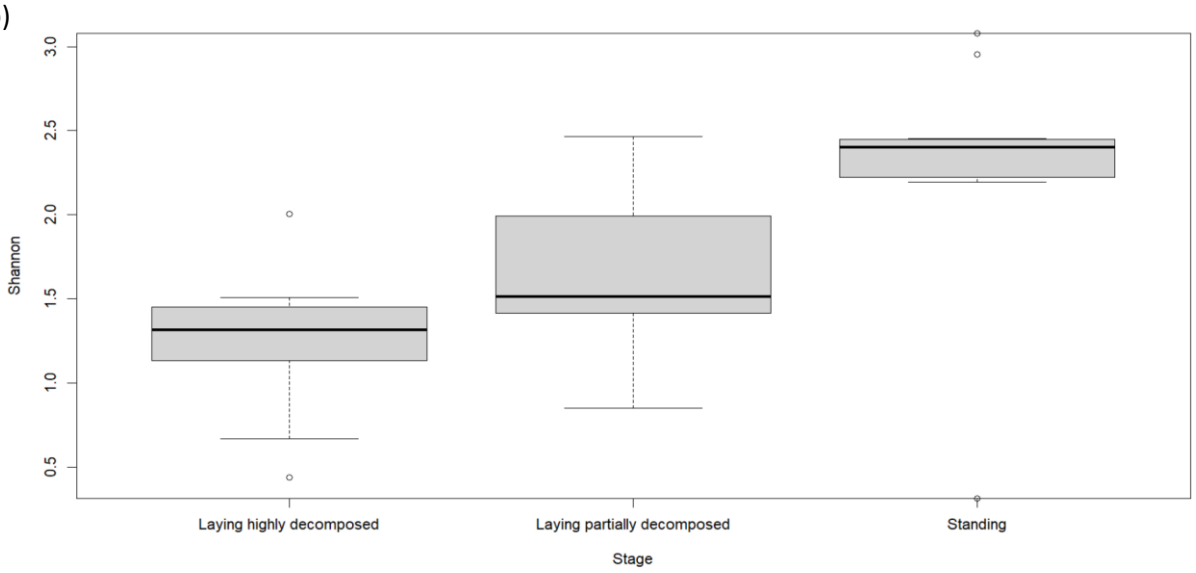


Figure 5. Various boxplots of alpha diversity indexes measure abundance and consistency in all three different groups of decay stage. (a) Boxplot of observed OTUs represent community richness, (b) Boxplot of Shannon diversity index represents diversity of species in the community and reflects the diversity of OTU in samples. Both figures (a + b) are based on 267 registered species in Villmyrane, Bø i Telemark.

3.4 Beta diversity

The principal coordinate analysis mostly separated the samples from standing to the samples from laying trees along the first axis (see Figure 6). Stages “Laying highly decomposed” and “Laying partially decomposed” are not so distinctly different from each other. The samples are ordinated closer to one another in a group. Four samples from the stage “Laying highly decomposed” formed a separate cluster on both axes. These samples were collected from a single tree (ID 8) in the stage “Lying highly decomposed” with barcodes 29, 30, 31 and 32. Those barcodes have strongly dominated fungal microbiome with distinguished yeast fungi (Saccharomycetes). The most common yeasts are *Wickerhamomyces bisporus, Kuraishia molischiana*. There are also a limited number of polypores, such as *Fomitopsis rosea and Mycoacia fuscoatra*.

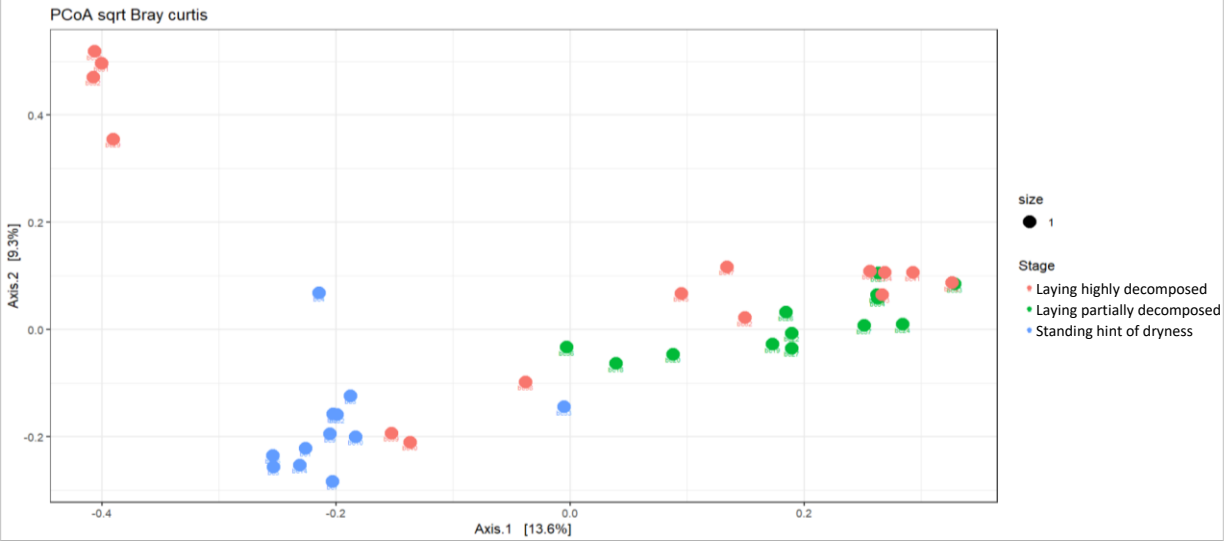


Figure 6. Principal-coordinate analysis (PCoA) scatterplot based on Bray Curtis dissimilarity matrix. Plots illustrate the distance between communities for each stage of decomposition. Groups: (Red) Laying highly decomposed, (Green) Laying partially decomposed, (Blue) Standing hint of dryness.

3.5 Functional guild

Figure 8 shows noticeable differences in guild between groups of decomposition stages. The greatest variation has stages “Standing hint of dryness” and “Laying partially decomposed”. Most common guild in all three decomposition’s stages are Plant saprotroph – Wood saprotroph (13) and Undefined Saprotroph – Wood Saprotroph (15). The central in the figures is a gradual increase in the number of functions from standing trees to

partially and highly decomposed trees in guild such as Ectomycorrhizal – Fungal Parasite (3), Endophyte – Undefined Saprotroph – Wood Saprotroph (8), Plant Pathogen – Undefined Saprotroph (11). Group 3 was classified in the family *Tricholomataceae* and included mostly the genus *Mycena.* Group 8 was classified in the family *Vibrisseaceae* and included mostly the genus *Phialocephala.* Group 11 was classified in the family *Mycosphaerellaceae* and includes genus such as *Phlebia* and *Acrodontium.* The "Plant Pathogen – Plant Saprotroph" (10) are prevalent in early stage of decomposition. Group 10 was classified in the family *Dermateaceae* and included mostly plant pathogen and saprobic on herbaceous and woody material, none of the registered genus or species dominates. Animal pathogen (1) stands out only in stage "Standing hint of dryness". Group 1 was classified mostly in the family *Cordycipitaceae* and included genus such as *Lecanicillium* and *Beauveria*.

1. Animal Pathogen
2. Animal Pathogen – Fungal Parasite-Undefined Saprotroph
3. Ectomycorrhizal – Fungal Parasite
4. Ectomycorrhizal – Fungal Parasite – Plant Pathogen – Wood Saprotroph
5. Ectomycorrhizal – Lichen Parasite – Lichenized – Plant Pathogen
6. Ectomycorrhizal – Wood Saprotroph
7. Wood Saprotroph
8. Endophyte – Undefined Saprotroph – Wood Saprotroph
9. Plant Pathogen
10. Plant Pathogen – Plant Saprotroph
11. Plant Pathogen – Undefined Saprotroph
12. Plant Pathogen – Wood Saprotroph
13. Plant Saprotroph – Wood Saprotroph
14. Undefined Saprotroph
15. Undefined Saprotroph – Wood Saprotroph

Standing hint of dryness | Laying partially decomposed | Laying highly decomposed

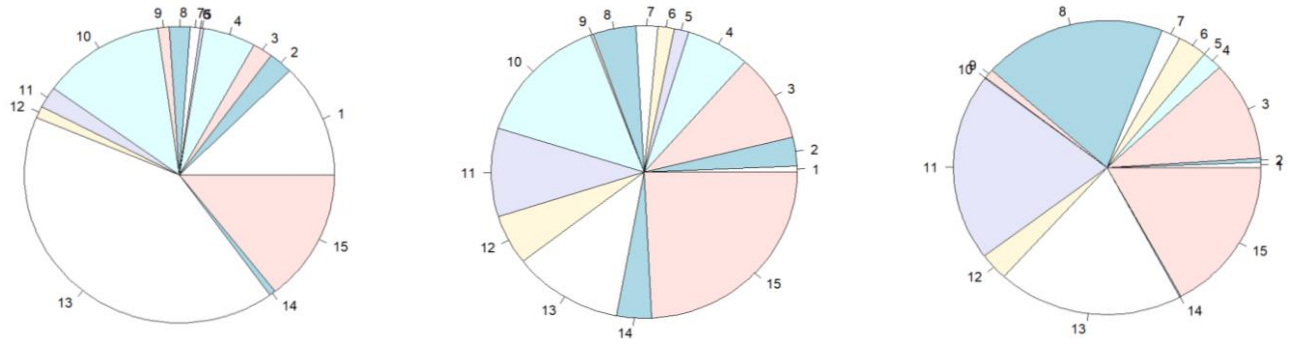


Figure 8. Pie charts illustrating the relative guild abundance according to different decomposition stages: "Standing hint of dryness", "Laying partially decomposed" and "Laying highly decomposed".

3.6 Red List assessment

Table 2. Norwegian Red List Assessment (Artsdatabanken, 2021) for all registered species in study area, distributed by decomposition stages; Standing hint of dryness, Laying partially decomposed, Laying highly decomposed. Red list categories: Near-threatened (NT), Vulnerable species (VU).

Red List assessment		NT	NT	NT	VU	VU	VU
Stage	Barcode	Fomitopsis Rosea	Phellopilus Nigrolimitatus	Phlebia Centrifuga	Rhodonia Placenta	Crustoderma Dryinum	Phlebia Subulata
Standing hint of dryness	bc 2	29					
	bc 10			11			
Partially decomposed	bc 19			32856			
	bc 20			20550			
	bc 22						11
	bc 24			13			
	bc 26				8350		
	bc 27					2928	340
	bc 57					55	
Highly decomposed	bc 29		56				
	bc 34		6768				
	bc 39						112
	bc 43	14					
	bc 62				37		

Out of all 31 registered polypores identifies by DNA, six species (see Table 2) are classified as near threatened or vulnerable on the Norwegian red list for species 2021 (Artsdatabanken, 2021). Of the assessed species, three are vulnerable and three are near threatened. The table highlights a noticeable increase in the number of red list species from standing to laying trees. The stage “Lying partially decomposed” contains four of the six species. It is worth noting that two of the red listed species (*Fomitopsis rosea* and *Phlebia centrifuga*) were detected in standing live trees.

4.0 Discussion

4.1 Succession of the fungal community

In this thesis I have studied the succession of the fungal community from live standing trees to dead well decomposed trees in an old-cultivated forest in Norway. As the present study encompassed, the forest has a heterogeneous structure that is covered by a wide variety of species. It can be emphasized with the result from the data collection related to one spruce tree which shows dominance of *P. centrifuga* in two extracted holes, while two others extracted holes shows dominance of species such as *F. pinicola* and *A. serialis*. Fungal diversity is relatively stable in the number of species found. The fungal species richness was relatively similar among stages “Laying partially decomposed” and “Laying highly decomposed”. There were nonsignificant differences, nevertheless Shannon index were highest during the early stage, this indicates less diversity (Weaver & Shannon, 1949); (Shannon, 1948) in live standing trees compared to dead well decomposed trees. There are similarities between mine results and what follows the findings of fructification pattern studies, which indicated that diversity is known to increase with decay progress (Fukasawa & Matsukura, 2021).

Beta diversity displayed highly similarity among stages “Laying highly decomposed” and “Laying partially decomposed”. The fungal species composition changes during substrate succession, most likely because of the chemical and physical changes during wood decomposition affecting the structure of fungal community and lead to changes in fungal species composition (vanderWal , Ottosson, & deBoer, 2014). Interestingly, the high values in shift community in stage laying highly decomposed comes of most likely strongly dominated fungal microbiome with yeast fungi (Saccharomycetes). Yeast was also present in other samples but not as prevalent, a possible explanation can be that environmental local temperatures that increase Saccharomyces species (Boynton, et al., 2021). All four extracted holes in that one tree registered the same, it can be suggested that the method works well, and the contamination is equal.

4.2 Functional guild

The current study underlines the trophic mode of the fungi vary at the different decay stages. More specifically, the FUNGuild analysis assigned the fungal species to the main trophic modes: animal pathogen, ectomycorrhizal, endophyte and saprotrophs (Figure 8). The results suggested that saprotrophs (plant, wood and undefined) proportion increase with increased decomposition of wood. The increase in dominance of wood saprotrophs, animal pathogen and wood endophytes. Wood saprotrophs included fungi present at the outset in low abundances as ectomycorrhiza and included mostly the genus *Mycena* which are considered as saprotroph-symbiotroph. Animal pathogen that included mostly genus such as *Lecanicillium* and *Beauveria* which are entomopathogenic fungus that reduce the rate of spread of hemlock woolly adelgid (Wickert, 2016). Wood endophytes that affect early community assembly and subsequent decay rates (Cline, et al., 2017). Mine findings show the opposite of previous study, that ectomycorrhizal fungi inhibit decomposition by competing with saprotroph fungi in later stages of decomposition (Fukasawa & Matsukura, 2021). Changes in functional characteristics were mainly very difficult to interpret as the data is usually based on a higher taxonomic rank (such as family). For example, I found 102 fungal ASVs using FUNGuild which cannot be annotated to any function by FungalTraits and confidence level.

4.3 Rare species

Many rare wood-inhabiting fungi are often linked to only later stages of wood decomposition (Rolstad & Storaunet, 2015). In contrast to previous studies, my results suggest that rare species are more common at later stages but are present at early stages. Species *F.rosea* and *P.centrifuga* are two rare species that were registered in stage “Standing hint of dryness” and their small amount of presence suggests that the drill hit exactly the right spot during sample collection. A greater number of rare species were observed in later stages of decomposition. As the present study encompassed registered rare species in early stage without presence of fruit-bodies can associated with other researchers suggest that production of fruiting bodies and spores is energy-intensive for the fungus, and many years can therefore pass between each time a fungus produces a fruiting body. Therefore, many fungi tend to reside as vegetative mycelium in wood and therefore do not develop fruiting bodies (Kubartova et al., 2012); (Hoppe, et al., 2014); (Jørund & Gjerde, 2003).

5.0 Conclusion

The main aims in the study were first to study the succession of the fungal community from living standing trees to dead well decomposed trees. Data from the study shows that trophic mode of the fungi vary at the different decomposition stages. There are good reasons to argue that saprotroph increases in proportion when the wood is more decayed. There are large amounts of saprotrophs in the stages where the trees are lying, which is typical for old culture forests. There are unrelated relationships between saprotroph and ectomycorrhizal.

Furthermore, the second main aim was to study the timing of establishment of rare polypore fungi that are used in monitoring of old growth forests. The hypothesis that many of the species that are rare in fruitbody surveys have the ability to establish early and can be found in all stages of decay was partly supported by the detection of two red listed species (*F. rosea* and *P. centrifuga*) in standing live trees. The findings from this study show that rare species are more common at later stages but are present at early stages. In terms of understanding the succession fungal community, this study may further develop knowledge about fungi species richness related to different stages of decomposition in old-cultured spruce forest.

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