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Forest management effects on aerial fungal biodiversity

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Contents

Abstract

Intensive forest management practices, including clear-cutting, have significantly impacted the structural composition of boreal forests over the course of several decades, affecting the forest biodiversity. However, the consequences of such intensive management practices remain unclear for many organismal groups. This study aims to compare the diversity of aerial fungal spores between near natural forests and previously clear-cut forests that have reached a late successional stage. My central hypothesis is that the aerial fungal diversity is higher in near natural forests compared to clear-cut forests. Moreover, I expect that increasing vegetation cover and amounts of deadwood will correlate positively with the aerial fungal diversity.

To test these hypotheses, the aerial fungal diversity was monitored using passive spore traps deployed across nine pairs of forest sites, each pair comprising one clear-cut (CC) forest and one near natural forest (NN). I placed two spore traps within each forest and conduced two sampling rounds. The first round spanned over a duration of two weeks, while the second round lasted for three weeks. Subsequently, DNA extracted from the spore filters was subjected to DNA-metabarcoding analysis to explore the fungal diversity.

Most of the detected fungi represented plant-associated fungi, probably to a large extent spread from the spruce trees. Although sampling round and climate, likely connected to plant phenology, had strongest impact, my findings indicate that clear-cutting also induce a shift in the aerial fungal community composition. Several of the detected fungal OTUs were strongly associated either with CC or NN forests. I observed a non-significant trend of higher fungal richness in the near natural forests in comparison to clear-cut forests. My results suggest that while clear-cut forestry may not reduce the aerial fungal richness *per se* when comparing mature forests, they do influence the species composition. My study indicates that spore-trapping coupled to DNA-metabarcoding is a promising technique to monitor fungal diversity, although it is not yet fully clear to what extent it represents the local versus the regional fungal diversity. Future spore-trapping studies should focus on to what extent the technique detects the local diversity only, and better understand how intensive forestry practices impact highly specialized rare and red-listed species.

Introduction

The boreal forest, spanning Northern Europe, Asia and North America, covers a major part of the northern hemisphere. Boreal forests are exhibiting a complex forest structure and dynamic that has developed over millennia, resulting in diverse habitats inhabited by native species in Fennoscandia (Kuuluvainen, 2009). Boreal forests offer a wide variety of habitats, giving rise to a wide variety of species (Baldrian, 2017). However, the structure of many boreal forests has undergone a drastic change due to forestry practices (Chaudhary et al., 2016). In Fennoscandia, forests and forestry have held cultural, economic, and ecological importance throughout history (Josefsson et al., 2010). Human influence on Norwegian forests can be traced back 5000 years, with selective logging being the primary harvesting strategy until approximately 1940 (Nygaard & Øyen, 2020; Aasetre & Bele, 2009). The early 1900s witnessed extensive forestry growth driven by population expansion, commercialization, and timber export. Stand-based forest management took over as the dominant forestry strategy after the 1940s. As a result, around 60% of Norwegian forests have been subjected to clear-cutting, making old-growth forests increasingly rare (Storaunet & Rolstad, 2020). Currently, only 1.7% of Norwegian forests remain unaffected by forestry activities (Storaunet & Rolstad, 2020). The general ecological condition of Norwegian forests falls below the threshold for good condition, and the continuation of current forest industry policies is predicted to exacerbate the forest's condition (Framstad et al., 2021).

The extensive clear-cutting has led to significant changes in forest structure and composition. The once-diverse old-growth forests, characterized by abundant biodiversity and large, old trees, have been transformed into more uniform forests with reduced biodiversity and even-aged trees (Linder & Östlund, 1998). Post-clear-cutting forests do not provide the same habitat characteristics in terms of dead wood abundance, tree size, and habitat connectivity, resulting in a less favorable environment for numerous species (Storaunet & Rolstad, 2020). The landscape resulting from clear-cutting exhibits a patchwork mosaic of even-aged forest stands (Aasetre & Bele, 2009). The consequences of such intensive forest transformations on biodiversity remain insufficiently understood for various organismal groups, as species responses are influenced by numerous biotic and abiotic factors, including temperature, moisture, disturbances like fires and insect invasions, species interactions, climatic events, and other variables (Baldrian, 2017). In general, unmanaged forests exhibit higher species

richness, greater dead wood abundance, and more diverse forest structures compared to managed forests (Boucher et al., 2015). The structural alterations caused by clear-cutting in boreal forests have resulted in a decline in forest biodiversity, with an estimated 1800 species already experiencing local extinction or becoming threatened in southern Finland (Kuuluvainen, 2009). Bowd et al. (2021) have demonstrated the negative impact of intensive forest management on plant-soil microbial community interactions. Valiente-Banuet et al. (2015) argued that species loss and the subsequent disruption of species interactions could have profound effects on ecological functions and ecosystem. Since many species are interdependent, the loss of one species may lead to the loss of several others, initiating a cascade of species loss. Species interactions serve as indicators of ecosystem health, as they provide valuable ecosystem services. Hence, species loss can lead to reduced ecosystem health (Valiente-Banuet et al., 2015).

Fungi play significant roles in the forest ecosystem, serving as saprotrophs, symbionts, and parasites (Stajich et al., 2009). Saprotrophs are important decomposers of dead organic matter (Peay et al., 2016); by recycling the components from dead organisms they make nutrients available for other organisms (Watkinson et al., 2016). Mycorrhizal fungi form mutualistic associations with plants, aiding in their establishment, growth, and enhanced nutrient uptake, including nitrogen, phosphorus, and water (Smith & Read, 2008). Lichens represent another symbiotic fungal group, characterized by a symbiotic relationship between fungi and algae or cyanobacteria (Watkinson et al., 2016). Endophytic fungi represent another abundant group, residing within plant tissues without harming the host plant (Blackwell, 2011). In contrast, parasitic fungi infect and cause harm to the plant host (Stajich et al., 2009). The fungal kingdom comprises an estimated 6.2 million species, the majority of which remain undiscovered (Baldrian et al., 2022). Given the vast diversity within this taxonomic group, with many undescribed species, predicting the impact of forestry on fungi becomes challenging. Additionally, various fungal groups, such as wood saprotrophic fungi and lichens, exhibit strong associations with old-growth forests owing to the abundance and diversity of trees and decaying wood (Juutilainen et al., 2014; Kuusinen & Siitonen, 1998).

Fruit bodies serve as pivotal life stages for numerous fungi, enabling the production and dispersal of spores through air, water, or vectors such as insects and animals (Hussein et al., 2013). The dispersal range of spores varies among fungal species and is influenced by various abiotic factors (Hussein et al., 2013), such as wind and precipitation (Martinez-Bracero et al., 2022). Peay and Bruns (2014) demonstrated that many fungi have limited spore dispersal, resulting in high local variation in species composition.

Accurately assessing the biodiversity of fungi in boreal forests presents a considerable challenge. Not all fungi form visible, macroscopic, fruiting bodies; some produce microscopic fruit bodies not visible with the naked eye, others make short-lived fruit bodies lasting for only a couple of days, while most of the early diverging fungal lineages, non-dikarya fungi, do not produce fruit bodies at all. To overcome these limitations, one approach involves aerial spore sampling to analyze and identify fungal species and to map the diversity of fungi e.g. (Aguayo et al., 2018). One effective method for species detection is DNA metabarcoding, which involves the application of DNA sequencing techniques to samples collected through spore traps. Abrego et al. (2018) demonstrated the efficacy of this approach in identifying actively reproducing fungal species. Other studies employing spore sampling have yielded valuable insights into spore dispersal (Peay & Bruns, 2014), fungal plant pathogen diversity (Chen et al., 2018) and airborne microbial biodiversity (Chen et al., 2018; Karlsson et al., 2020; Martin-Sanchez et al., 2021). Spore sampling methods from the air can involve active techniques such as volumetric samplers (Tordoni et al., 2021) or passive spore traps (Redondo et al., 2020). Comparing active and passive spore traps, Redondo et al. (2020) found that they shared between 55% to 61% of fungal species. The authors hypothesized that the differences in dispersal abilities among spores captured by the two methods may account for this disparity. Furthermore, they suggested that passive spore traps are more effective in capturing local variation, whereas active spore traps are better suited for capturing spores from surrounding habitats. Spore trapping offers valuable insights into fungal life cycles, factors influencing fungal biodiversity, spore dispersal distances, habitat requirements, and more (Martinez-Bracero et al., 2022). However, limited research has investigated the utility of spore sampling in assessing the impact of forestry on fungal biodiversity.

By integrating spore sampling with DNA-based methods, it is possible to obtain valuable information about fungi's hidden lifestyles (Baldrian, 2017). Continuous advancements in DNA-based technologies are significantly enhancing our understanding of fungal communities and their ecological roles. DNA metabarcoding involves the amplification of a specific DNA marker, typically the rDNA ITS region (Schoch et al., 2012), which is subsequently analyzed through high-throughput DNA sequencing (Nilsson et al., 2019). The vast amount of data generated by high-throughput sequencing enables comprehensive exploration of fungal communities across diverse ecosystems (Nilsson et al., 2019).

The primary objective of this master thesis is to investigate the impact of clear-cutting on fungal biodiversity in southeastern Norwegian boreal forests. This is achieved through a comparative analysis of aerial fungal diversity between two forest types: near natural (NN) forests that have never undergone clear-cutting and mature forests that were previously clear-cut (CC) and has now reached a late successional stage. The assessment of fungal diversity and community composition in spore trap samples is conducted using DNA metabarcoding.

The research questions and corresponding *hypotheses* addressed in this master thesis are as follows:

1. How does clear-cut forestry affect the aerial fungal spore community? In this regard*, I hypothesize that the near-natural forest hosts an overall higher fungal diversity than clear-cut forests and that the composition of spores in air varies between the two forest types.*
2. How does variation in forest structure, encompassing tree size, tree species, and tree age, impact fungal communities? *I expect that forests with a more complex forest structure contain a more diverse fungal species composition.*
3. How does the quantity and quality of dead wood influence fungal biodiversity? *I expect to find a more diverse fungal community in the forests with higher quantity and quality of dead wood.*
4. How does the distribution of fungal functional guilds vary across forest types? *I expect more wood-decomposer fungi and lichens in near natural forests compared to clear-cut forests.*

Material and methods

Study design and sampling

A total of 72 passive spore traps were deployed across nine forest sites located in southeastern Norway (Fig. 1a) between September and November 2021. Each site consisted of a pair of forests: a near-natural forest (NN) and a mature forest that had previously undergone clear-cutting and had reached a late successional stage (CC). Forest pairs were selected based on similar characteristics, including topography and soil properties. Within each forest, a 15 x 15 m plot was designated, with the bottom-left corner serving as the reference point (coordinates X_0Y_0). Two spore traps were installed at positions $X_0Y_{7.5}$ m and $X_{15}Y_{7.5}$ m, respectively (Figure 1b). The spore traps utilized a plastic funnel equipped with a metal sieve to prevent the entry of large debris. The funnel was placed within a cylindrical container with a removable bottom, which contained a grid. A filter composed of Cyclopore polycarbonate membrane (1 µm pore size and 47 mm diameter; VWR) was positioned on the grid to capture fungal spores and fragments. To ensure stability, the spore trap was affixed to a wooden stick at an above-ground height of approximately 1.15 m (Figure 1c).

Prior to deployment in the forest, the spore traps underwent thorough cleaning (first washed with soap, then sprayed with 70% ethanol and rinsed with distilled water) and were stored in sterile plastic bags to prevent contamination. The placement of filters within the spore traps was carried out in the forest using gloves and sterile tweezers. Two filters were sampled from each spore trap. The first filter remained in the spore trap for approximately two weeks (14-15 days), while the second set of filters was exposed in the trap for about three weeks (20-23 days). After collecting the first spore filter, and before adding the second spore filter, the spore traps were washed with 70% ethanol and distilled water. Following collection, the filters were folded in half to prevent the spores to stick to the inside of the bag and individually stored in small ziplock bags at -20 °C until further processing.

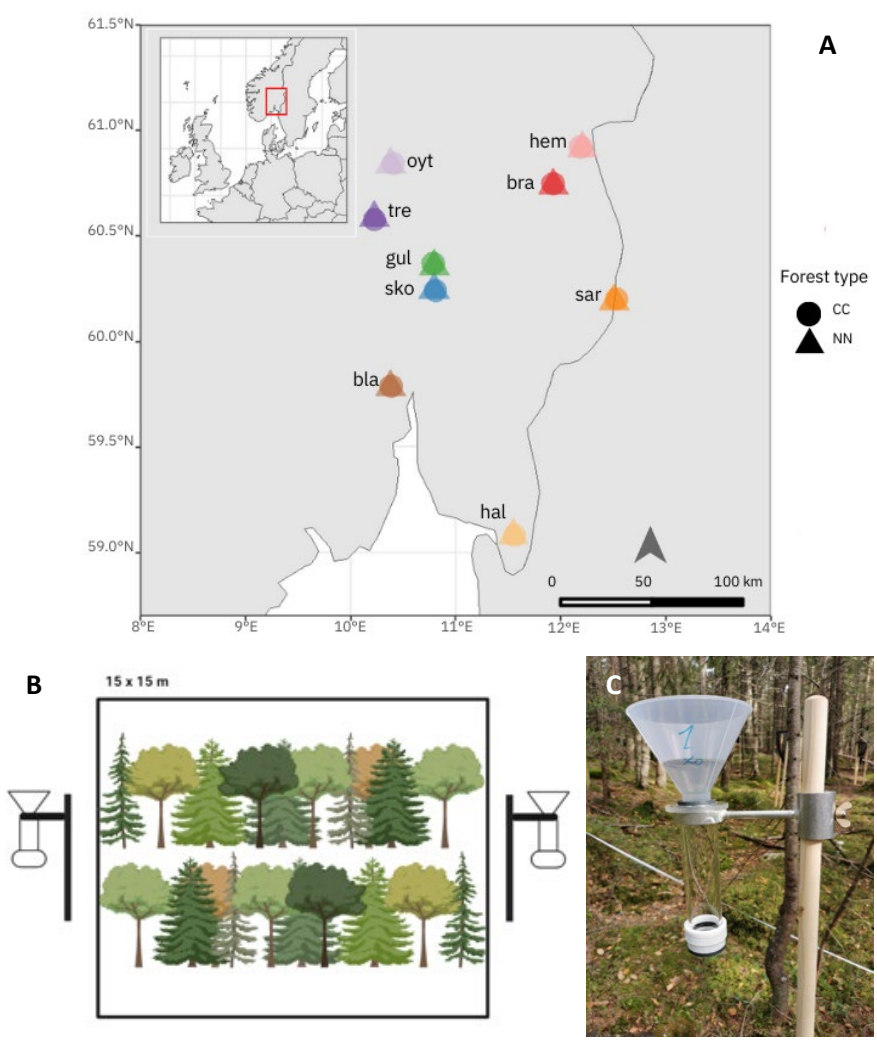

Figure 1. Study sites and sampling design for comparing fungal biodiversity. a) *The map illustrates the geographical locations of the study sites in southeastern Norway. The nine pairs of sites, listed from north to south, include Hemberget (hem), Øytjern (oyt), Braskreidfoss (bra), Tretjerna (tre), Gullenhaugen (gul), Skotjernfjell (sko), Särkilampi (sar), Blåfjell (bla), and Halden (hal). Each location consists of two forest types. The near natural (NN) forests are denoted by triangles, while the clear-cut (CC) forests are represented by circles.* ***(b)*** *In each forest, two passive spore traps are placed at the same elevation on each side of a 15 x 15 m plot.* ***(c)*** *The passive spore traps consists of a tube with a removable sieved bottom and a funnel inserted on top. A metal grid is placed in the funnel to prevent debris from falling into the tube. The spore trap is fastened with a metal fastening to a stick and placed about 1.15 m above the ground. A cyclopore membrane filter of 1 µm is placed on the removable sieve in the bottom of the tube.*

Molecular methods

DNA was extracted from the 72 filters using a modified CTAB-chloroform protocol. Initially, any large organic matter such as spruce needles was removed from the filters. Subsequently, the filters were placed in 50 ml Falcon tubes containing 4 ml of 0.2 M Tris-HCl buffer. These Falcon tubes were then subjected to shaking at 600 rpm for 15 minutes in an incubator set at room temperature (VWR INCU-Line). Additionally, vortexing was performed vigorously for 10 seconds at high speed to detach the spores and wash the filters. Before further processing, the filters were removed and the Falcon tubes containing the buffer were spun for 30 min at 3500 rpm in a centrifuge (Thermo Scientific Heraeus Multifuge X3R) at 6 °C. For each sample, the top 1 ml of the supernatant was discarded, 2 ml were preserved as a backup and stored at -20 °C, and the remaining 1 ml was utilized for DNA extraction.

To the 1 ml of supernatant for DNA extraction, 100 µl Tris-HCl with 0,1% Chitinase was added and vortexed. The tubes were then centrifuged at 3500 rpm for 1 minute (Eppendorf centrifuge 5425), and 600 µl of the supernatant was transferred from the Falcon tubes to 2 ml Eppendorf tubes, carefully avoiding any residual debris. In the 2 ml Eppendorf tubes, two tungsten beads and 600 µl of CTAB (cetyltrimethylammonium bromide) with 2% β-Mercaptoethanol were added, resulting in a total volume of 800-1000 µL in each tube. The samples were grinded using a Tissue Lyser (Qiagen TissueLyser II) for 1 min at 30 hz. Following this, the Eppendorf tubes were incubated in a heating block (VWR Thermomixer Comfort) at 90 °C for 5 minutes and then placed in liquid nitrogen. This freezing and heating step was repeated three times. Afterwards, the tubes were incubated at 65 °C for 60 minutes with agitation at 800 rpm for 20 seconds, interspersed with 10 minute intervals.

Once the Eppendorf tubes had cooled down, 800 µl of chloroform was added, and the tubes were vortexed and centrifuged at 3500 rpm for 15 minutes. From the supernatant, 700 µl were transferred to a 1.5 ml Eppendorf tube containing 400 µl of cold isopropanol. The tubes were inverted and incubated overnight at 4 °C. The tubes were centrifuged at 3500 rpm for 10 minutes, the isopropanol was carefully decanted, and the resulting pellets were washed twice with 300 µl of cold 70% ethanol. Each wash involved centrifugation at 3500 rpm for 2 minutes. The ethanol was removed, and the tubes were placed in a heating block at 50 °C, with open lids to evaporate residual ethanol droplets. Finally, 60 µl of Tris-HCl buffer was used to resuspend the DNA, which was subsequently stored at -20 °C.

Library preparation and sequencing

DNA quantification was performed using the Qubit 2.0 Fluorometer from Thermo Fisher Scientific. The amplification of the ITS2 region was carried out using the forward fITS7 primer and the reverse ITS4 primer (Ihrmark et al., 2012). To ensure reproducibility, nine PCR replicates were included. Unique molecular identifiers were incorporated into all PCR primers to enable sequence identification. PCR reactions were conducted under standardized conditions. Each of the PCR reactions performed in 25 µl volume consisted of 3 µl DNA template and 22 µl master mix: 12.6 µl milli-Q H2O, 2.5 µl AmpliTaq Gold Buffer 10x, 2.5 µl $MgCl_2$, 1 µl BSA 20 mg/ml, 0.2 µl 25 nM dNTPs, 0.2 µl AmpliTaq Gold polymerase, 1.5 µl 10 µM forward primer and 1.5 µl 10 µM reverse primer.

PCR reactions were run with initial denaturation 98 °C for 30 s, followed by 30 cycles of denaturation at 98 °C for 10 s, primer annealing at 55 °C for 15 s and elongation 72 °C for 15 s. Followed by a final elongation 72 °C for 5 min, and then cooling down to 10 °C until collected. PCR products were controlled with gel electrophoresis on 1% Agarose gel.

The PCR amplicons were normalized across samples using SequalPrep Normalization Plate Kit, 96-well (Thermo fisher scientific) following the manufacturer's protocol. DNA concentrations were measured with Qubit BR DNA Assay (Thermo Fisher scientific) and pooled (3.53 ng/µl). The ITS2 region (200-350 pb) was paired-end sequenced on the Illumina MiSeq platform at StarSEQ (Mainz, Germany).

Bioinformatics

The bioinformatics analysis for processing the sequences was conducted on the Saga Cluster, provided by UNINETT Sigma2 –the Norwegian Infrastructure Services for High Performance Computing and Data. Firstly I demultiplexed the paired-end forward and reversed sequences using CUTADAPT (Martin, 2011), which also removes primers and tags. CUTADAPT options did not allow miss-match with primer tags, with a minimum overlap of 26 bp between forward and reverse primers and the minimum length per sequence was set to 100 bp and no indels were permitted. Then the *demult* files with very low read numbers were removed. Further processing was done using the DADA2 pipeline (Callahan et al., 2016) in the R statistical environment 4.2.1 to correct errors and dereplication.

To account for the length variability that occurs in ITS sequences, ITS1 BAND_SIZE was set to 32 and maxEE=c(1,1), which allows one error in the overlapping sequences in each direction. DADA2 then merges the forward and reverse sequences, with a 50-nucleotide minimum overlap was set to merge the sequences together. DADA2 then generates an amplicon sequence variant (ASV) table, where all chimeric sequences were checked and removed. The ASVs were then clustered into operational taxonomic units (OTUs) using VSEARCH (Rognes et al., 2016) , with similarity set at 97%, to account for the relatively high interspecific variability in ITS region and curated to correct for over-splitting of OTUs with LULU (Frøslev et al., 2017).

To taxonomically annotate the OTU table, the ITS 2 sequences were blasted against the UNITE database (Nilsson et al., 2018). The blast returned five hits per OTU and the query coverage was set to 80%. The UNITE blast returned 1758 out of 2274 OTUs with fungal taxonomy. The 516 sequences that weren't taxonomically annotated with UNITE were blasted against NCBI database (Johnson et al., 2008). Blasting through NCBI was done manually and returned 5 hits per OTU and query coverage at 90%. Taxonomical annotation was decided based on identity score. The NCBI blast returned an additional 167 hits. The final OTU table contained 1925 taxonomically annotated OTUs. FunGuild (Nguyen et al., 2016b) was used for assigning functional guild and verified and completed by Professor Klaus Høiland. In total, after quality filtering, the dataset consisted of 5 700 115 reads. 2 835 830 reads belonging to clear-cut (49.75%) and 2 864 285 belonging to near-natural (50.25%) forests respectively. Per sample there was a mean of 80 283 reads. The mean number of reads per sample is 79 563 in near-natural (NN) and 81 023 in clear-cut (CC). Sample S051.CC_tre_1_s1 was removed due to a low (376) number of reads, totalling 71 samples analysed. To account for sequencing depth the OTU table was rarified to the lowest sample read number i.e. 23 552 reads, using the *rrarefy* function in R from the package VEGAN (Oksanen et al., 2020). The OTU table was standardized by making the number of reads per OTU proportional for each sample.

Statistical analysis

All the statistical analyses were conducted using R 4.2.1 environment (R Development Core Team, 2022). The packages I used in the analysis were VEGAN (Oksanen et al., 2020) for ordination diagrams and variation partitioning, METACODER (Foster et al., 2017) and PHYLOSEQ (McMurdie & Holmes, 2013) for visualizing OTU abundance and plotting taxonomy, TIDYVERSE (Wickham et al., 2019) and GGPLOT2 (Wickham, 2016) were used to model and visualize plots.

To investigate the effects of different environmental variables on the aerial fungal biodiversity, several plot-specific variables were either collected in the field or obtained from modelled datasets (Table S1). Vegetation variables obtained in the field refer to the number of trees in the plot, tree species diversity is a Shannon diversity index, total vegetation cover measured in certain areas in the plot, total Shannon diversity of the vegetation measured in certain areas, the GNMDS1 values calculated from vegetation and the GNMDS2 values calculated on vegetation. The dead wood variables are the number of dead wood logs, unique combinations of dead wood, dead wood functional dispersion and dead wood functional

evenness. The Norwegian metrological institute provided modelled datasets with local weather precipitation and temperature data for the sampling period. Modelled dataset with annual mean temperature and annual precipitation were provided by Horvath et al. (2019). For all spore trap filters, number of conifer needles were counted and the percental coverage of debris was measured by taking high-resolution pictures which were analyzed using the ImageJ software (Schneider et al., 2012). Detailed explanations of all environmental variables are listed in the supplementary information (Table S1).

A rarefaction curve was made using the *rarecurve* function from VEGAN (Oksanen et al., 2020). When calculating richness, the rarified dataset was transformed to presence/absence data and the abundances were then summed up at the sample-level. To see the effect of site variables and environmental variables on richness, ANOVA (analysis of variance) and regression models were calculated using the built-in function *aov* in R. A GLM (generalized linear model) model was fitted using the *glm* function from the built-in R package STATS. Forward selection using CCA (canonical correspondence analysis) was performed for variation partitioning of the different site and environmental variables using the *cca* function from VEGAN. Ordination plots were conducted using NMDS (non metrical multidimensional scaling) using the function *monoMDS* from the VEGAN package. Environmental variables were fitted to the ordination plot using the function *envfit* frm the package vegan. To see the effect of the different variables on community composition PERMANOVA (Permutational multivariate analysis of variance using distance matrices) analysis, using the *adonis2* function form the VEGAN package was performed. Functional guild bar charts were conducted on relative abundance (in %) from the entire dataset. Indicator species analysis were performed using the *multipatt* function from the INDICSPECIES package (De Caceres & Legendre, 2009). For portraying the indicator species analysis, a CCA was performed on forest type with sampling round and location partitioned out. The indicator species were then highlighted in the CCA plot and colored by functional guilds.

Results

The effect of forestry on fungal species richness

In total, after quality filtering, the dataset ended up with 1925 OTUs in 71 samples containing 5 700 115 reads. The near-natural (NN) forests were represented with 36 samples and the clear-cut (CC) with 35 samples. There was an average of 80 283 reads per sample and 514 OTUs per sample. The rarefaction curves (Figure 2) indicates that, although many samples tend to a plateau, none of them exhibited complete flattening. The rarefaction curve suggests that a substantial portion of the fungal biodiversity was captured in the samples.

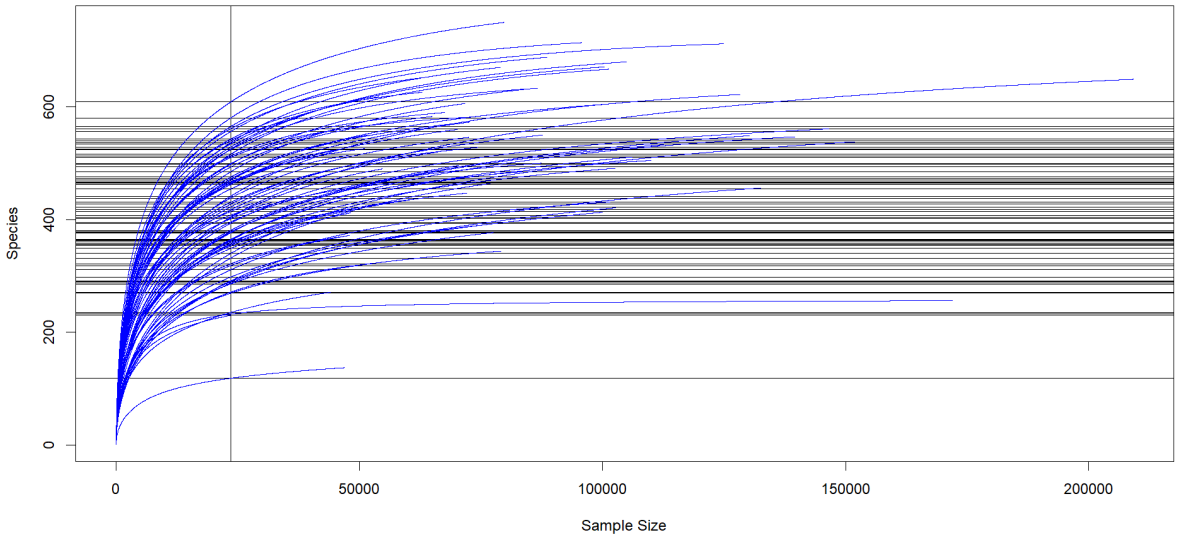

Figure 2. Rarefaction curve illustrating the relationship between sample size and observed fungal richness. *This figure presents the association between sample size and the observed fungal richness, measured in terms of the number of fungal species, within the sampled fungal community. Each separate sample is represented by blue lines. The minimum sample size recorded in this analysis was 23 552.*

Comparison between the two forestry management strategies revealed a slightly higher richness of aerial fungal biodiversity in the near-natural forests compared to the clear-cut forests (ANOVA, p-value = 0.23; Figure 2), while the sampling round had a clear effect on fungal species richness (ANOVA, p-value = 1.06e-07 ***; Figure 2). Richness and Shannon diversity per sample were highly correlated (Pearson index = 0.97, p-value = 2.2e-16 ***), and I therefore chose to focus only on richness. In the first sampling round, fungal richness ranged from 459 to 536 in the NN forests and from 419 to 516 in the CC forests. In the second sampling round, richness ranged from 277 to 384 in NN and from 311 to 384 in CC.

Neither dead wood variables nor vegetation variables had significant effects on the fungal richness (Table 1). However, both average precipitation and average temperature (Table 1), which were both measured during the sampling period, had a significant effect on fungal richness.

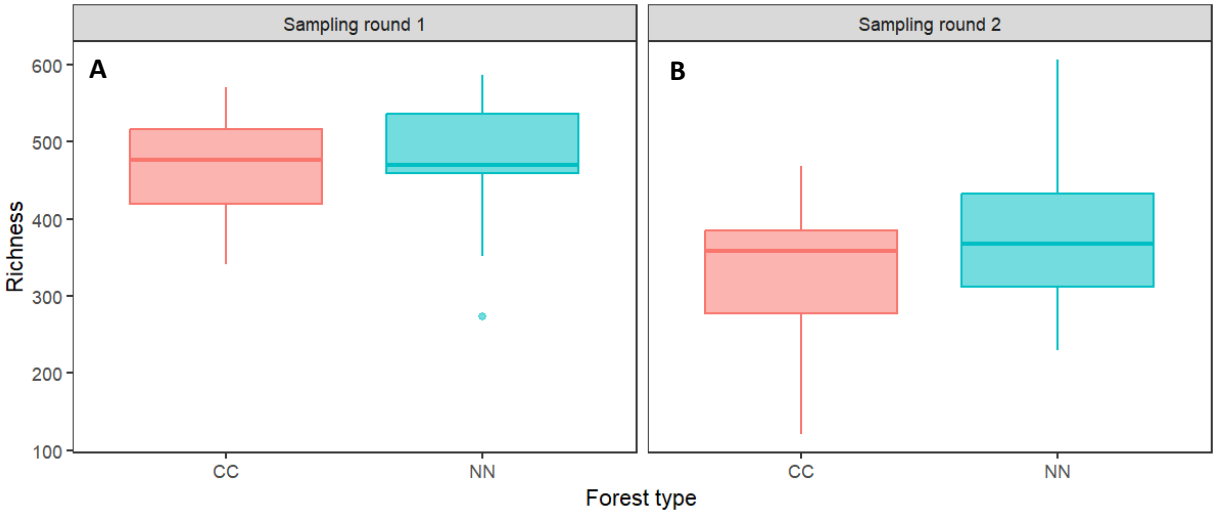

Figure 3. Boxplot representation of fungal species richness between forest types. ***(a)*** *represents sampling rounds 1, where the filters were placed out for 2 weeks, while* ***(b)*** *corresponds to sampling rounds 2, where the filters were out for 3 weeks. The boxplots depict the comparison of forest types, with clear-cut (CC) forests represented in orange and near natural (NN) forests depicted in turquoise, in terms of richness measured by the number of OTUs.*

A generalized additive model (GAM) was used to evaluate the joint effects of the environmental variables (Table 2). This model indicated that fungal richness was slightly lower in NN compared to CC although not statistically significant (Table 2). Sampling round 2 displayed a significantly lower richness compared to sampling round 1. In line with this, the model showed that more precipitation and higher temperature were significantly related to a higher fungal richness (Table 2). The GAM analysis also revealed that the number of dead wood logs had a significant negative influence on fungal richness.

Table 1. Analysis of variance (ANOVA) investigating the impact of variables on fungal richness (number of OTUs). *This table presents the results of an ANOVA analysis examining the influence of site, environmental and weather variables on richness. Richness was computed by converting the read counts into presence-absence data and aggregating the number of OTUs accordingly for each sample. The analysis encompassed a set of environmental variables, with only statistically significant environmental variables being included and reported in this table. Forest type is displayed, even though it's not significant since it's the focal factor.*

Variables	**P-value**
Forest type	0.23
Sampling round	1.06e-07 ***
Average precipitation	0.00025 ***
Average temperature	0.00028 ***

Table 2. Generalized additive model (GAM) on variables for fungal richness. *The Generalized Additive Model (GAM) was employed to investigate the relationship between fungal richness and various predictor variables. These variables were site location, forest type, sampling rounds, and a set of environmental variables. The model underwent a stepwise selection process, whereby variables were sequentially eliminated based on their respective p-values, resulting in the table of only statistically significant variables. Forest type is displayed, even though it's not significant, since it's the focal factor.*

Variable	Coefficient	P-value
Intercept (Location - Bla, Forest type - CC, Sampling round 1)	1058.15	0.00014 ***
Location – Bra	-91.34	0.13
Location – Gul	-184.84	0.0077 **
Location – Hal	-43.74	0.3
Location – Hem	-183.45	0.048 *
Location – Oyt	-105.12	0.18
Location – Sar	-26.96	0.6
Location – Sko	-79.36	0.1
Location – Tre	82.17	0.16
Forest type – NN	-4.66	0.81
Sampling round 2	-273.53	0.0032 **
Average precipitation	14.34	0.0071 **
Average temperature	-62.46	0.032 *
Number of dead wood logs	-1.9	0.00094 ***

Drivers of fungal community composition

When looking at compositional variation in an NDMS ordination analysis (Figure 4), it turned out that forest type (NN versus CC) had a small but significant effect on the aerial fungal community composition (Table 3). The forest types tend to be grouped together in the ordinations within their respective locations across the two sampling rounds (Figure 4 b and c). In addition, vegetation variables, like vegetation GNMDS2 (gnmds2_veg), total vegetation Shannon diversity (Tot_H) and number of trees in the plot (nr.of.trees.in.plot) were correlated with NMDS1, tentatively affecting the aerial fungal community (Table 4a).

Sampling round, as well as the weather variables average temperature and precipitation, strongly correlated with NMDS2 (Table 4a). The number of dead wood logs significantly correlated with the compositional variation (Table 4a). There was also a strong and significant location effect (Table 4), which became more visible when analyzing the two sampling rounds separately (Figure 4 b and c). A strong latitudinal trend can be seen in Figure 4 b and c, where latitude is correlated to NMDS1. The location effect may include unmeasured environmental variation connected to the latitudinal gradient.

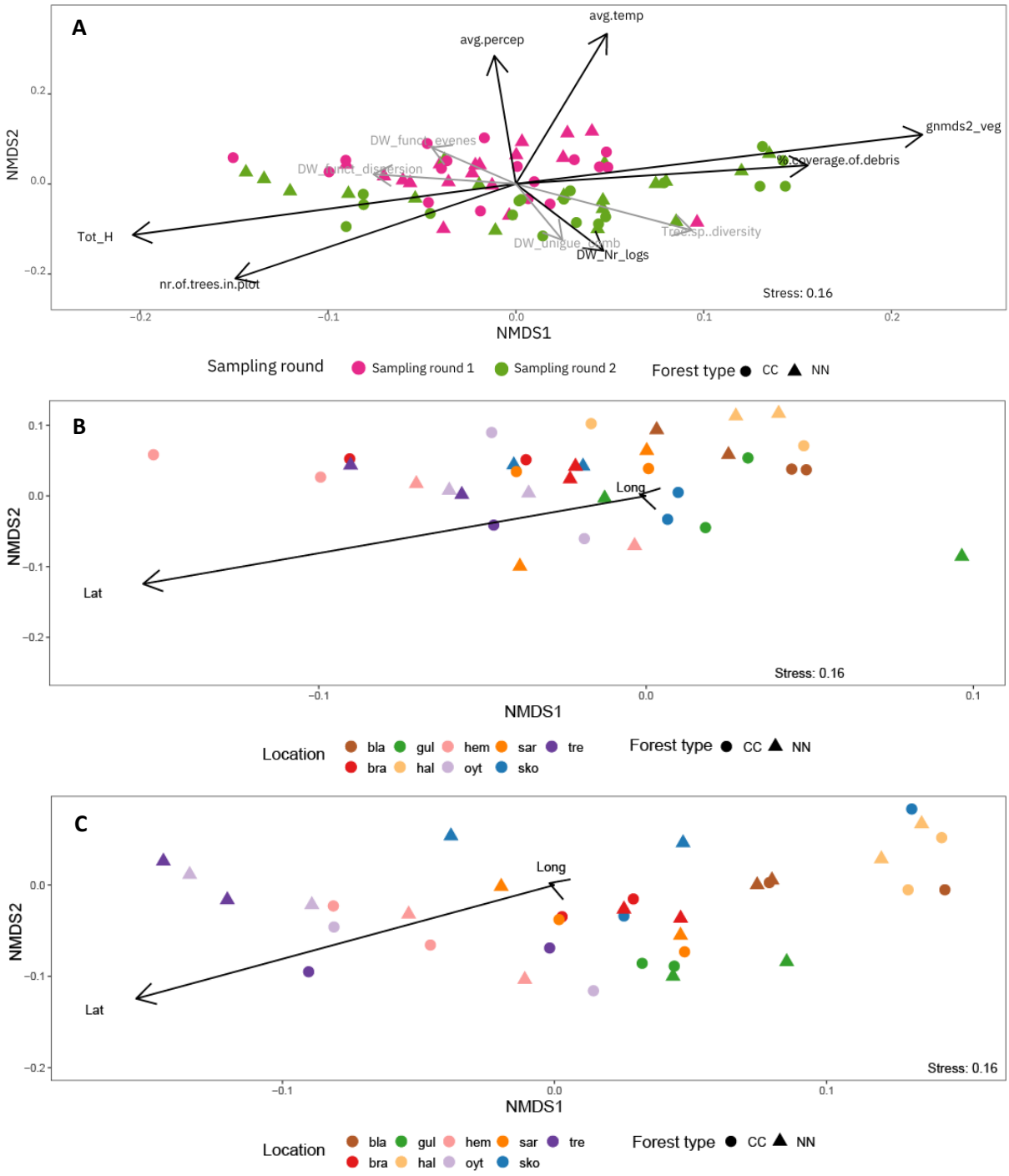

Figure 4. Nonmetric multidimensional scaling (NMDS) ordination plots displaying the sampled fungal community. *This figure showcases the NMDS ordination plots depicting the fungal community composition. Clear-cut (CC) samples are represented by circles, while near natural (NN) samples are depicted by triangles. Each shape corresponds to a specific sample.* ***(a)*** *The NMDS plot encompasses both sampling rounds. Sampling round 1 is color-coded in pink, whereas sampling round 2 is color-coded in green. Environmental variables that significantly influence community composition are indicated by black arrows, while non-significant variables are depicted in gray. NMDS plots focusing solely on* ***(b)*** *Sampling round 1 samples (2 weeks sampling time) and* ***(c)*** *Sampling round 2 samples (3 weeks sampling time), with arrows illustrating latitude and longitude to demonstrate the geographical trend in the ordination. The NMDS values for latitude and longitude is divided by two to make the plot more readable. The latitude arrow exhibits statistical significance, whereas the longitude arrow does not. The variables in the analysis are zero skewed. Stress value = 0.16.*

Table 3. Permutational multivariate analysis of variance (PERMANOVA) on the effect of environmental variables on fungal community composition. *This table presents the results of PERMANOVA conducted to evaluate the impact of environmental variables on fungal community composition. The analysis was performed on all variables simultaneously. Variables that were not found to be statistically significant were not included in the table.*

Variables	R2	P-value
Location	0.3	0.001 ***
Sampling round	0.11	0.001 ***
Forest type	0.023	0.003 **
Total Shannon diversity	0.015	0.05 *
Number of trees in plot	0.024	0.003 **
Total vegetation cover	0.014	0.046 *
Tree species diversity	0.015	0.031 *
Vegetation GNMDS2	0.015	0.031 *
Average temperature	0.017	0.009 **
Average precipitation	0.027	0.001 ***
Residual	0.41	
Total	1	

The joint impact of the environmental variables on community composition was assessed using variation partitioning through canonical correspondence analysis (CCA), with forward selection of variables. All environmental variables, except two dead wood variables (dead wood functional evenness and dead wood functional dispersion), exhibited a significant effect on community composition in this analysis (Table 4). Collectively, the examined variables accounted for 43.75% of the variation in the entire dataset (Table 4). Forest type alone explained 2.01% of the variation only, while location and sampling round accounted for 22.03% and 4.82%, respectively. The vegetation variables collectively explained 13.19% of the variation, with vegetation Shannon diversity having the highest explanatory power at 3.90%. The dead wood variables, as a whole, contributed to 5.20% of the variation, with the number of dead wood logs explaining 2.72%. Weather variables accounted for 7.53% of the total variation.

Table 4. Variation partitioning of fungal community composition analyzed by canonical correspondence analysis (CCA) with OTU data and environmental variables. *This table presents the results of variation partitioning analysis conducted through Canonical Correspondence Analysis (CCA) to assess the contribution of environmental variables to the variation in fungal community composition. The variation explained by the environmental variables was modeled using a forward selection approach. Only statistically significant variables were included in the variation partitioning analysis.*

Variable	Model	Constrained proportional inertia (%)	P-value
Location	OTU ~Location	22.03%	0.001 ***
Sampling round	OTU~Samplinground + Condition(Location)	4.82%	0.001 ***
Forest type	OTU~Forest_type + Condition(Location + Samplinground)	2.01%	0.013 *
Total[1] variation explained by site variables	OTU~ForestType+Location+Sampling round	28.86%	0.001 ***
Total Shannon diversity on vegetation	OTU ~ Total Shannon diversity on vegetation	3.9%	0.001 ***
Vegetation GNMDS2	OTU~ Vegetation GNMDS2 + Condition(Total Shannon diversity on vegetation)	2.5%	0.008 **
Number of trees in plot	OTU~ Number of trees in plot + Condition(Total Shannon diversity on vegetation+ Vegetation GNMDS2)	2.7%	0.001 ***
Total[2] vegetation cover	OTU~Total vegetation cover + Condition(Total Shannon diversity on vegetation+Vegetation GNMDS2+Number of trees in plot)	3.1%	0.001 ***
Tree species diversity	OTU~ Tree species diversity + Condition(Total Shannon diversity on vegetation+Number of trees in plot+Total vegetation cover)	3%	0.002 **
Total[3] variation explained by vegetation	OTU~ Tree species diversity + Condition(Total Shannon diversity on vegetation+Number of trees in plot+Total vegetation cover	15.24%	0.001 ***
Number of dead wood logs	OTU ~Number of dead wood logs	2.72%	0.01 **
Unique combinations dead wood	OTU~ Unique combinations dead wood + Condition(Number of dead wood logs)	2.49%	0.007 **
Total[4] variation explained by dead wood	OTU~ Unique combinations dead wood + Number of dead wood logs	5.2%	0.002 **
Average temperature	OTU~AverageTemperature	4.42%	0.003 **
Average precipitation	OTU~AveragePrecipitation + Condition(AverageTemperature)	3.11%	0.001 ***
Total[5] variation explained by weather	OTU~AverageTemperature+ AveragePrecipitation	7.53%	0.001 ***
Total variation explained	OTU~Total [1]+ Total[3] + Total[4]+ Total[5]	46.45%	0.001 ***

Taxonomic and functional composition

The taxonomic composition analysis revealed a slightly higher abundance of Ascomycota compared to Basidiomycota (Figure 5a). Among the most prevalent classes identified were Leotiomycetes, Tremellomycetes, and Dothideomycetes. When comparing the 15 most abundant orders in the CC forests and NN forests, a considerable similarity is observed (Figure 5b). However, there were some orders that exhibited greater abundance in CC, such as Cantharellales and Triblidiales, while others, like Capnodiales and Phacidiales, were more prominent in NN. Nevertheless, no fungal orders turned out, in the metacoder (Figure 5a), to have a significantly association to either forest type (Wilcoxon Rank Sum test).

Out of the total 1925 original OTUs, functional guilds were assigned to 1072 (55.7%). Among the different trophic groups, plant-associated fungi emerged as the dominant group (Figure 6), followed by yeasts and wood saprotrophic fungi. A significant difference in the proportion of lichen sequences per sample was observed between CC and NN (t-test, p-value = 0.029 *), with lichens exhibiting higher abundance in NN. None of the other functional guilds displayed a significant difference between forest types. However, there was a general trend that all trophic groups showed higher abundances in NN compared to CC (Figure 6), except for ectomycorrhizal fungi and fungi without functional guild.

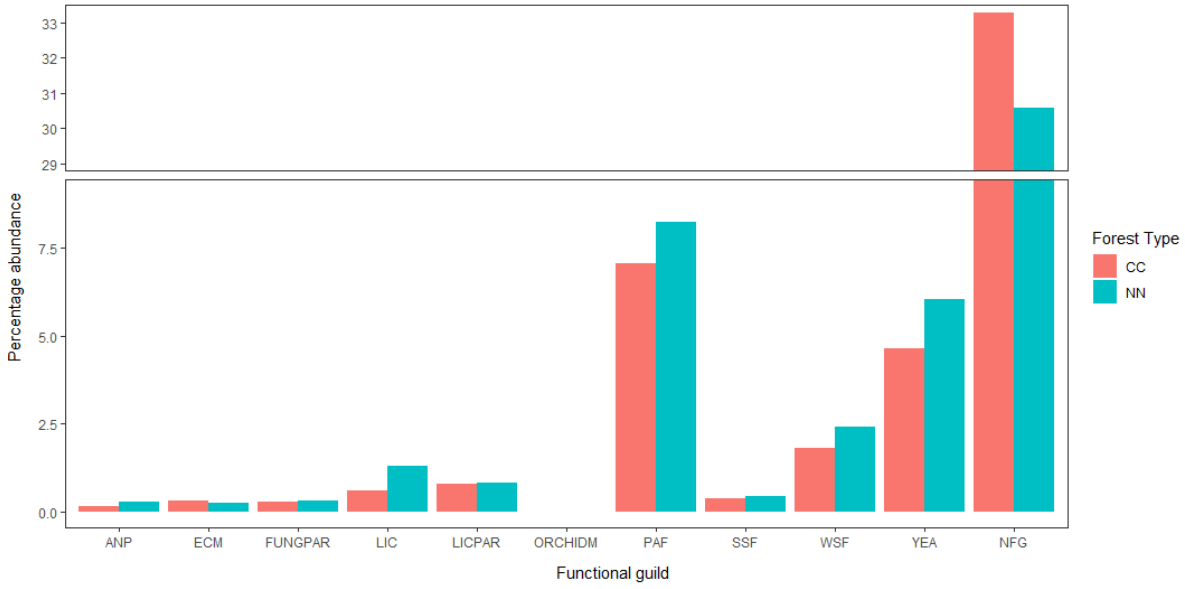

Figure 6. Comparison of fungal distribution in functional guild groups between two forest types, near natural and clear-cut. *This bar chart illustrates the distribution of fungi across ten functional guild groups in two forest types. The clear-cut (CC) forest type is visually represented by orange, while the near natural (NN) forest type is depicted in turquoise. The functional guild groups are abbreviated as follows: WSF = Wood saprotrophic fungi, SSF = Soil saprotrophic fungi (including dung fungi), ECM = Ectomycorrhizal fungi, LIC = Lichens, PAF = Plant-associated fungi (encompassing pathogens, endophytes, and saprotrophs), ANP = Animal pathogens, YEA = Yeasts and dimorphic fungi, NFG = no functional guild. The percental abundance of each functional guild group is calculated based on the entire dataset.*

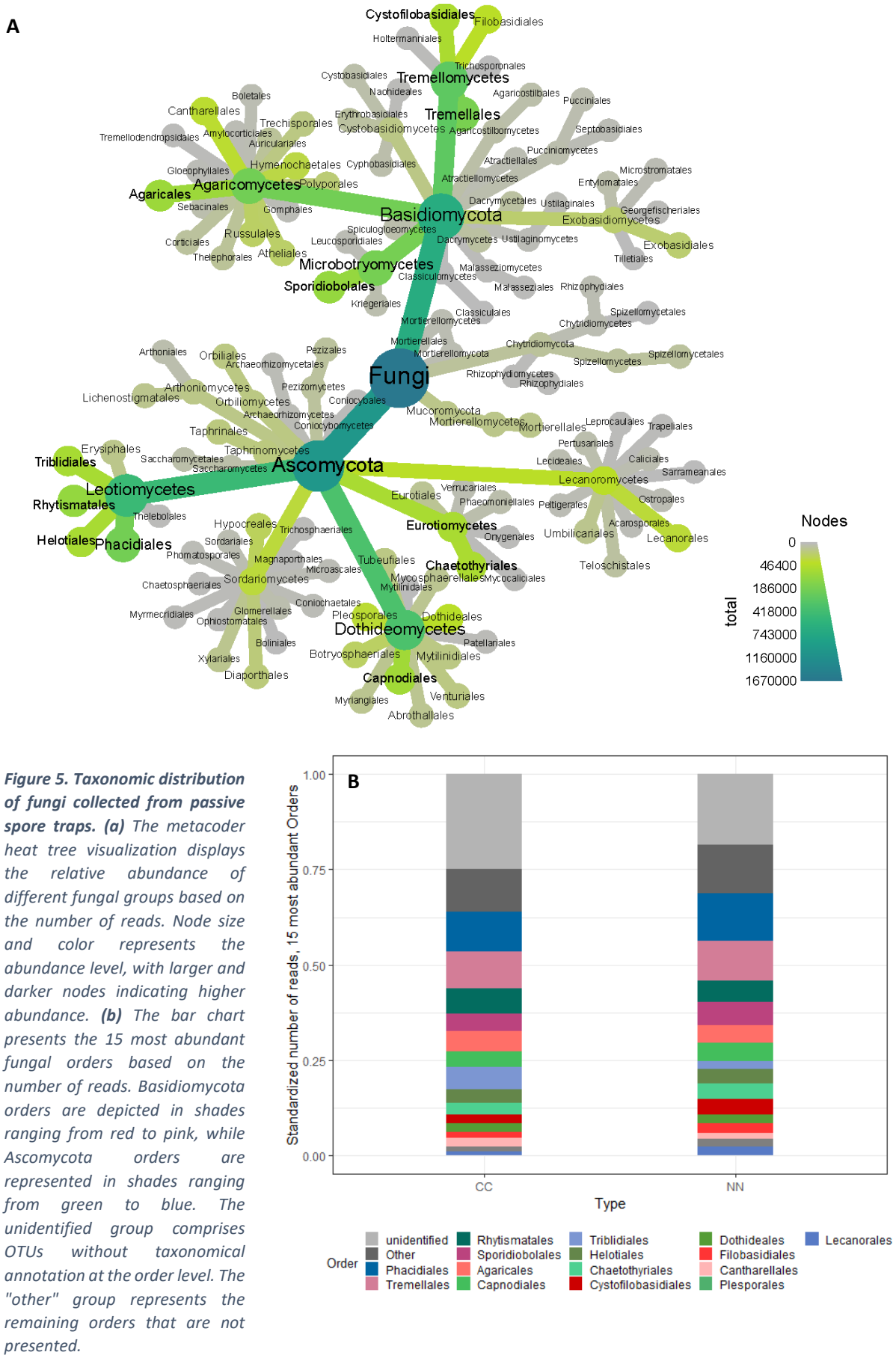

Figure 5. Taxonomic distribution of fungi collected from passive spore traps. (a) *The metacoder heat tree visualization displays the relative abundance of different fungal groups based on the number of reads. Node size and color represents the abundance level, with larger and darker nodes indicating higher abundance.* ***(b)*** *The bar chart presents the 15 most abundant fungal orders based on the number of reads. Basidiomycota orders are depicted in shades ranging from red to pink, while Ascomycota orders are represented in shades ranging from green to blue. The unidentified group comprises OTUs without taxonomical annotation at the order level. The "other" group represents the remaining orders that are not presented.*

Indicator species analysis

A total of 79 indicator species were identified, with 49 in NN and 30 in CC (Figure 7), having a significant association to either NN or CC (Permutation test). In CC, the indicator species comprised 3 plant-associated fungi, 6 yeasts, 1 wood saprotrophic fungi, and 20 species without functional guild. In NN, the indicator species included 7 plant-associated fungi, 1 yeast, 5 lichens, 6 wood saprotrophic fungi, 1 lichen parasite, 1 animal pathogen, 2 soil saprotrophic fungi, and 26 species without functional guild. The supplementary material provides an overview (Table S2) of the indicator species assigned to a specific functional guild.

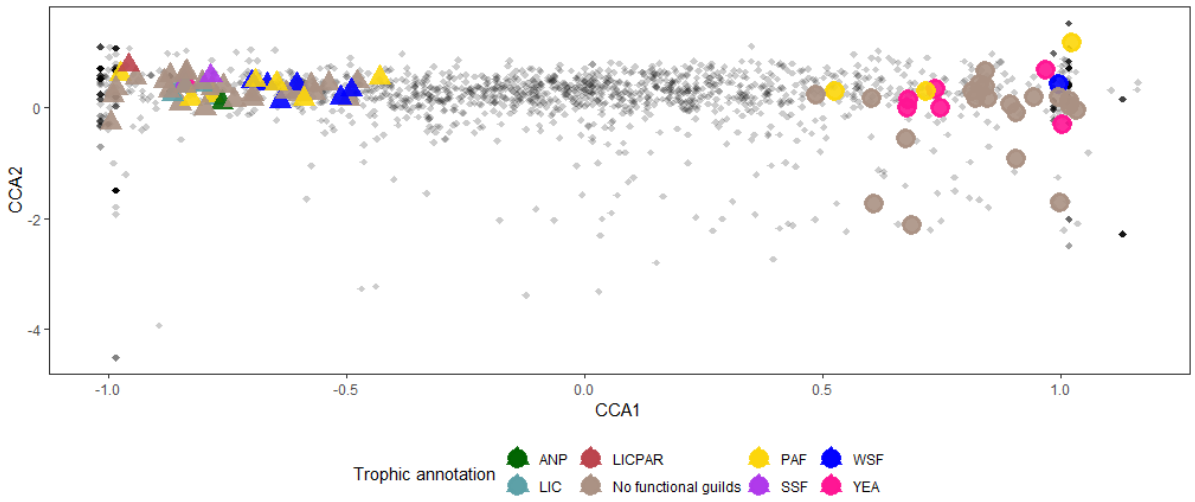

Figure 7. Indicator species analysis, comparing near natural and clear-cut forests, displayed on canonical correspondence analysis (CCA) with sampling round and location partitioned out, to display the effect of forest type*. This figure showcases the results of an Indicator Species Analysis overlaid on a CCA performed on forest type, with sampling round and location variables conditioned out. Indicator fungal species, identified using the IndicSpecies package in R, are color-coded based on their functional guild. Near-natural (NN) forests are represented by triangles, while clear-cut (CC) forests are depicted by circles. Species that were not significantly more present in either forest type in the indicator species analysis are visualized as smaller gray dots in the background. CCA1 axis explains 14.41% of the variation, while CCA2 axis explains 7.75%.*

Discussion

Intensive forestry practice has led to a big decline in near natural forests in Norway (Storaunet & Rolstad, 2020). As a response to this decline, habitat loss has been observed in near natural forests (Framstad et al., 2021), leading to a negative trend for species dependent on specific habitats in near natural forests (Kuuluvainen, 2009). A better understanding of how intensive forest management practices affect fungal biodiversity can help us better understand how to preserve these species. The main goal for this study was to investigate the effect of intensive forestry on fungal biodiversity, using spore sampling combined with DNA metabarcoding. I wanted to see how the overall patterns in species richness, community composition and taxonomically composition were affected by intensive forestry and which environmental factors were driving the differences. Considering the structural differences between these types of forests (Tomao et al., 2020), I expected to find a difference in fungal biodiversity between near natural forests and previously clear-cut forests.

The effect of forestry on fungal species richness

Contrary to my hypothesis, the results did not reveal a significant difference between NN and CC forests in terms of aerial fungal richness. However, a trend was observed, indicating higher richness in the NN forests. Previous studies comparing fruit body occurrences on dead wood in highly managed forests to old-growth forests have shown a higher saprotrophic fungal richness in unmanaged forests (Abrego & Salcedo, 2014; Dvořák et al., 2017; Juutilainen et al., 2014). However, it is worth noting that other studies have reported similar or even higher fungal richness in managed forests compared to unmanaged forests (Blaser et al., 2013; Oria-de-Rueda et al., 2010; Runnel & Lõhmus, 2017). Blaser et al. (2013) conducted a study comparing managed coniferous, managed deciduous, and unmanaged deciduous forests, and found a lower number of fungal species in unmanaged forests. However, they attributed this difference to the relatively short time since management abandonment (10-30 years). Similarly, Oria-de-Rueda et al. (2010) investigated Mediterranean forests and examined whether artificial reforestation could develop fungal communities similar to those found in natural stands. They revealed that artificial reforestation could indeed provide fungal diversity and production comparable to that of natural forest stands. Furthermore, Runnel and Lõhmus (2017) sampled polypores and compared species between old and harvested forest stands in

Estonia. They found that most polypores in old forests were also present in harvested forests, except for a few rare species occurring exclusively in old-growth forests.

Similarly, for ectomycorrhizal (ECM), Spake et al. (2016) observed that old clear cut forests, which had reached a late successional stage (ca. 90 years), exhibited similar fungal ECM richness compared to unmanaged forests. This suggests that the diversity of ECM fungi can be restored in late successional stage, in this case 90 years. Spake et al. (2016) worked with ectomycorrhizal sporocarps and sequenced fungal DNA from root tips in oak-dominated forests. In line with their results, I can speculate that similar fungal richness between forest types in my study result from CC forests reaching a late successional stage.

It is important to acknowledge that these abovementioned studies relied on fruit body surveys, which can yield different results compared to those obtained from spore sampling (Castaño et al., 2017). Spore trapping might potentially detect species in the clear-cut forests that are not surveyed in fruit body inventories, which could explain the lack of significant differences observed between forest types in terms of fungal richness. Castaño et al. (2017) also found that not all the species producing fruit bodies were registered in the sequenced spore sample data. In future studies researcher can gain a more comprehensive understanding of the fungal community and the processes influencing its composition, by combining fruit body surveys with spore sampling.

Another aspect affecting fungal spores' ability to disperse is their shape and size (Calhim et al., 2018). The spore size, shape and ornamentation affect the spores ability to reach their desired substrate and are often correlated to the functional guild. Hussein et al. (2013) suggest that spore morphology and the aerodynamic properties are important for airborne dispersal. Hirst et al. (1967) found that atmospheric turbulence was a major contributor for spore dispersion, as well as wind shear, precipitation, and surface deposition. Using spore sampling could therefore give a skewed picture of the aerial fungal community as some spores are transported far and will more easily deposit on the filter, compared to other fungal groups.

The significant variables affecting fungal richness in this study were the weather variables, sampling round and location. It has been found that the fungal spore abundance is highly affected by local weather patterns (Crandall and Gilbert (2017). Tordoni et al. (2021), using high-throughput sequencing data from spores in Italy, found a lower abundance of spores in

the autumn compared to the spring/summer, which they explained by a lower temperature. These results are in line with the results obtained here, with a lower fungal richness (number of OTUs) in the second sampling round, likely due to colder weather.

Drivers of fungal community composition

While richness was influenced by fewer variables, community composition was found to be affected by multiple variables, including site characteristics, vegetation, dead wood, and weather variables. The canonical correspondence analysis (CCA) revealed that these variables collectively accounted for 46.45% of the observed variation in community composition. Other than effects of site variables, vegetation variables emerged as the primary drivers, suggesting that vegetation plays a crucial role in shaping the aerial fungal community composition. This observation aligns with the finding that a significant proportion of the detected fungi were classified as plant-associated based on functional guilds.

Forest type significantly influenced the fungal community composition, as demonstrated by the CCA analysis and the PERMANOVA analysis. These findings are consistent with those of Varenius et al. (2016) who conducted sporocarp surveys and analyzed ectomycorrhizal roots using restriction fragment length polymorphism (RFLP). Their study revealed an overall difference in the community composition of ectomycorrhizal (ECM) fungi between a natural pine forest and a 50-year-old managed pine forest. Similarly Goicoechea et al. (2009), observed differences in the composition of ECM communities between clear-cut and unmanaged Spanish beech forests.

After accounting for site variation (location, sampling round and forest type), vegetation variables emerged as the strongest predictors of fungal community variation, surpassing both dead wood and weather variables. This finding highlights the crucial role of vegetation in shaping the fungal community. A study by Redondo et al. (2020), supports this belief, as they concluded that vegetation is a driving factor in fungal spore deposition. However, it is important to note that their study compared coniferous forests, deciduous forests, and agricultural fields, which exhibit substantial differences in vegetation types compared to the present spruce-dominated forests. Another study conducted by Nguyen et al. (2016a) on Norway spruce needles in mature European forests demonstrated that tree species diversity affects the fungal community significantly. Their findings are in line with my study, where the

vegetation variable, tree species diversity, was significant both in the CCA and the PERMANOVA analyses.

While dead wood variables explained less variation than site, vegetation, and weather variables, two specific dead wood variables: (1) number of dead wood logs and (2) unique combinations of dead wood were found to significantly influence community composition in the CCA analysis. However, these variables did not yield significant results in the PERMANOVA analysis. Overall, these results suggest that both the quantity and diversity of dead wood may act as drivers of fungal community composition. A studie have also demonstrated a positive correlation between the amount and diversity of dead wood and fungal community composition (Olou et al., 2019). Despite the study by Olou et al. (2019), being performed in a tropical ecosystem, the authors suggest that the influence of dead wood diversity on wood-inhabiting fungal communities may be a general pattern worldwide.

When looking at each sampling round separately, the ordination analyses revealed there was a strong geographical latitudinal pattern in fungal community composition. Peay and Bruns (2014a) showed that fungal spore dispersal limitations can lead to high variation in local fungal community structure. Additionally, phenological differences in vegetation across locations likely play a role in this latitudinal trend. It is expected that the more northern localities are further ahead in the vegetation period due to the generally lower temperatures (Shi et al., 2014).

As mentioned earlier there is a strong connection between vegetation and aerial fungal communities (Redondo et al., 2022). A similar trend is seen by the location variable. However, the effect of location is much stronger than the effect of sampling round on community composition. This difference may be attributed to the relatively short time interval (2-3 weeks) between sampling rounds compared to the significant geographic gradient from south to north, which encompasses substantial climate and weather variations. Karlsson et al. (2020) observed significant variation in fungal communities both between and within locations, which they attributed to seasonal and geographical differences. They revealed that local landscape and seasonal variation shape the microbial community in the air. However, it is difficult to pinpoint the exact parameters encompassed by the location variable, as some of the variation explained by location may be attributed to unrecorded environmental variables in this study.

Taxonomic and functional composition

Wood saprotrophic fungi and lichens exhibited relatively higher abundance in NN forests compared to CC forests. In one study on Estonian forests, Runnel and Lõhmus (2017) observed that many wood saprotrophic fungi occurred both in old growth and managed forests, but there were some rare species that only occurred in old growth forests. A study from the Italian Alps shows that lichens prefer old growth forests over managed forests (Nascimbene et al., 2010), in correspondence with the results in my study. Lichens were the only functional guild being significantly more abundant in NN. Plausible explanations for this could be due to older trees in NN, which serve as vital habitats for epiphytic lichens. The same pattern was seen in a study by Kuusinen and Siitonen (1998), which demonstrated a similar trend of increased abundance of epiphytic and epixylic lichens in old-growth forests, studied in *Picea abies*-dominated boreal forests in southern Finland, when compared with managed forests.

While there was a similar trend for plant-associated fungi to be more abundant in NN forests, this difference was not statistically significant. Notably, plant-associated fungi represented the largest functional guild group, probably spread by surrounding spruce trees. The indicator species analysis indicated that NN forests contained a greater number of plant-associated fungi with a significantly stronger association to the forest type compared to CC forests. Among the plant associated indicator species, the majority consisted of plant pathogens and endophytes, with several of them exhibiting a close association with spruce. Fodor and Hâruța (2022) assert the significance of fungal pathogens in terms of biodiversity, emphasizing their intricate interactions, particularly with trees. With such complex interactions, a more diverse forest and vegetation structure may consequently harbor a broader array of plant-associated fungi. Consistent with the findings from Nguyen et al. (2016a), a study on the needle mycobiome sampled from Norway spruce in mature forests in Europe, some of the pathogenic fungal genera identified in my study included *Chrysomyxa* (rust pathogens of Norway spruce), *Taphrina* (plant pathogen) and *Ramularia* (plant pathogen).

Spore sampling as a study method

Spore sampling combined with high throughput sequencing has been shown to provide extensive insight into the present aerial fungal community (Banchi et al., 2018; Tordoni et al., 2021). This method has proven to be highly effective for monitoring aerobiology and has found diverse applications, including pathogen surveillance and health surveys (Martinez-Bracero et al., 2022). Further, as discussed above, Castaño et al. (2017) has shown that combining spore trapping and fruit body surveys yield a complementary result.

The rarefaction curves generated in this study demonstrate that the sampling effort has successfully captured a substantial portion of the aerial fungal community, resulting in a representative dataset. However, it is important to acknowledge that passive spore sampling, despite its efficacy, has certain limitations. The entire process, from field sampling to DNA isolation in the laboratory, high-throughput sequencing at the sequencing center, and data processing, introduces potential risks of contamination, false positives, and PCR bias (Aguayo et al., 2018). Furthermore, primer bias and incomplete or biased databases can impact the results (Abrego et al., 2018), as shown by the presence of a high number of unknown species in my analysis.

Conclusion

The primary objective of this thesis was to investigate the impact of intensive forest management on aerial fungal biodiversity. The findings do not suggest that that the practice of clear-cutting necessarily reduces the overall number of fungal species in the forest, however, it does alter the composition of the fungal community. Despite maintaining a relatively high fungal richness after forest management, there is a potential loss of important species associated with old growth forests. However, it is challenging to conclude that there is a higher overall fungal biodiversity in the NN forests based on the results alone. Taken together, the findings of this study indicate that community composition could serve as a better measure of the impact of forest management, compared to richness.

To further validate these findings, future studies should consider incorporating both spore sampling and fruit body surveys to obtain a more comprehensive understanding of the fungal community. Additionally, conducting spatial and temporal sampling throughout the year would provide insights into the seasonal dynamics and how the fungal community changes over time. It would also be beneficial in the future to investigate the effects of intensive forest management on rare and red-listed fungal species, considering their potential vulnerability to forestry practices.

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Supplementary information

Table S1. Description of environmental Variables, data collection, and data sources used in analysis of aerial fungal biodiversity. *This table provides an overview of all environmental variables used in the study, comparing aerial fungal biodiversity in near natural and clear-cut forests, including details on how they were collected and the sources from which the data were obtained.*

Variables	Abbreviation	How were they collected and/or calculated?	Source
Number of trees in the plot	Nr..of.trees.in.plot	The data was collected by directly counting the number of trees on-site.	Norskog
Tree species diversity	Tree.sp.diversity	The tree species present in the plot were identified and recorded during the data collection process. The Shannon diversity index was calculated based on the species composition data. It measures the species richness and evenness within a given area.	Collecte by: Norskog Calculated by: Author
Total vegetation cover	TotalVeg	The total vegetation cover was quantified by assessing the proportion of a certain area in the plot covered by vegetation.	Johan Asplund, NMBU Jenni Norden, NINA
Total Shannon diversity of the vegetation	Tot_H	The Shannon diversity index was calculated based on the composition of the entire vegetation community, considering all species present.	Johan Asplund, NMBU Jenni Norden, NINA
Number of dead wood logs	DW_Nr_logs	The number of dead wood logs present in the field was determined by direct counting during the data collection process.	Johan Asplund, NMBU Jenni Norden, NINA
Unique combinations of dead wood	DW_unique_comb	Unique combinations of dead wood is a measure of the functional richness of dead wood, which was assessed by identifying and quantifying the unique combinations of tree species, decay stage, and size. This provides information on the diversity and variety of functions represented by the dead wood.	Johan Asplund, NMBU Jenni Norden, NINA
Dead wood functional dispersion	DW_funct_dsipersion	To assess the functional dispersion of dead wood, a measure based on a multidimensional functional space of log characteristics was utilized. It takes into account the relative abundance of species and calculates the average distance of each log to the centroid in the functional space.	Johan Asplund, NMBU Jenni Norden, NINA
Dead wood functional evenness	DW_funct_evenes	The functional evenness of dead wood was evaluated using a measure based on a multidimensional functional space of log characteristics. This measure considers the relative abundance of species and quantifies the regularity or uniformity of points in the plot, indicating how evenly distributed the logs are in the functional space.	Johan Asplund, NMBU Jenni Norden, NINA
Average precipitation	Avg.percep	Precipitation data was modeled using gridded climate data with 1 x 1 km with a daily measurement resolution. Which provides information on precipitation	The Norwegian metrological institute

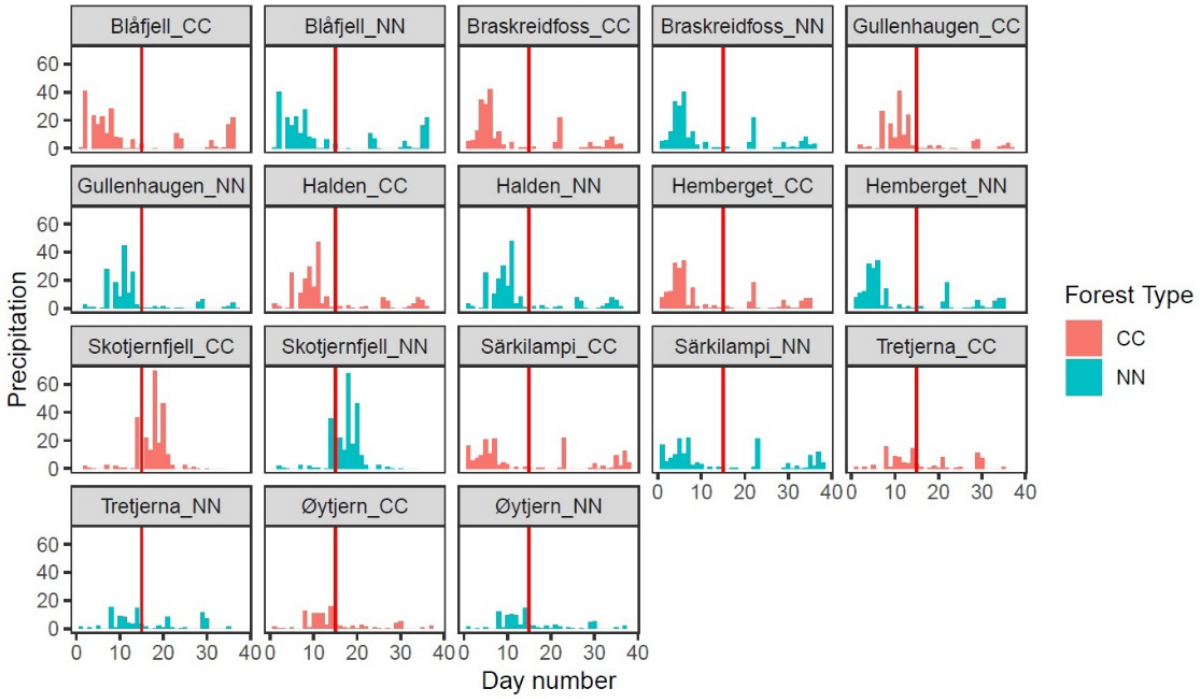
		patterns in the separate sampling rounds. The precipitation data was averaged over each sampling round.	
Average temperature	Avg.temp	Temperature data was modeled using gridded climate data with 1 x 1 km with a daily measurement resolution. Which provides information on temperature patterns in the separate sampling rounds. The temperature data was averaged over each sampling round.	The Norwegian metrological institute
GNMDS 1 vegetation	Gnmds1_veg	GNMDS analysis was performed on the vegetation data. The first axis was derived, which represents the primary dimension of variation and patterns in the vegetation community.	Johan Asplund, NMBU Jenni Norden, NINA
GNMDS 2 vegetation	Gnmds2_veg	GNMDS analysis was performed on the vegetation data. The second axis was derived, which represents an additional dimension of variation and patterns in the vegetation community.	Johan Asplund, NMBU Jenni Norden, NINA
Longitude	Long	The spatial coordinates of specific locations or points of interest were recorded using a Global Positioning System (GPS) device during fieldwork. This allows for accurate geolocation and mapping of the study area.	Ecoforest
Lattitude	Lat	The spatial coordinates of specific locations or points of interest were recorded using a Global Positioning System (GPS) device during fieldwork. This allows for accurate geolocation and mapping of the study area.	Ecoforest
Annual mean precipitation	Annual.precip.avg	Modeled annual mean precipitation, with 100 m x 100 m resolution.	Peter Horvath, NHM (Horvath et al., 2019)
Annual mean temperature	Annual.temp.avg	Modeled annual mean temperature with 100 m x 100 m resolution.	Peter Horvath, NHM (Horvath et al., 2019)
Number of needles on filter	Nr.of.needles	Number of needles were counted in the laboratory as part of the data analysis process.	By author
% coverage of debris on filter	%.coverage.of.debris	High-resolution images of all filters were captured, and the debris cover was quantified using the image analysis software ImageJ. This allowed for precise calculations of the proportion of filter area covered by debris.	By author
Heterogeneity of tree species	Heterogenity.tree.sp	The tree species present in the field were identified and recorded during the data collection process. The heterogeneity of tree species was calculated, which quantifies the diversity and variation in tree species composition within the study area.	Collecte by: Norskog Calculated by: Author
Number of days out	Nr..of.days.out	Registered while conducting fieldwork.	By author
End day number	End.day.nr	Registered while conducting fieldwork.	By author
Start day number	Start.day.nr	Registered while conducting fieldwork.	By author

Table S2. Indicator species analysis results. *This table presents the results from the indicator species analysis conducted on the fungal community from air samples obtained from near natural (NN) and clear-cut (CC) forests. The table includes the indicator value, p-value, forest type, and functional guild for each species. Only species with functional guild are included in this table. In addition to the species presented in the table, there were 46 species without functional guild, with 26 species belonging to NN forests and 20 species belonging to CC forests.*

Species	Indicator value	P-value	Forest type	Functional guild
Taphrina americana	0,49	0,041*	CC	PAF
Gloeotinia sp.	0,478	0,003**	CC	PAF
Taphrina padi	0,412	0,045*	CC	PAF
Amyloxenasma grisellum	0,378	0,031*	CC	WSF
Phenoliferia glacialis	0,633	0,005**	CC	YEA
Buckleyzyma phyllomatis	0,602	0,03*	CC	YEA
Piskurozyma cylindrica	0,557	0,03*	CC	YEA
Kockovaella mexicana	0,47	0,032*	CC	YEA
Heterocephalacria sp.	0,414	0,015*	CC	YEA
Heterocephalacria sp.	0,409	0,032*	CC	YEA
Lecophagus sp.	0,646	0,032*	NN	ANP
Lecidea nylanderi	0,747	0,001***	NN	LIC
Japewia subaurifera	0,676	0,032*	NN	LIC
Violella fucata	0,63	0,02*	NN	LIC
Ochrolechia microstictoides	0,625	0,002**	NN	LIC
Violella fucata	0,511	0,021*	NN	LIC
Abrothallus cetrariae	0,437	0,049*	NN	LICPAR
Pseudodidymella minima	0,641	0,037*	NN	PAF
Stagonospora bicolor	0,637	0,007**	NN	PAF
Dothiorella sp.	0,614	0,024*	NN	PAF
Hymenoscyphus subsymmetricus	0,531	0,034*	NN	PAF
Botryosphaeriaceae sp.	0,527	0,015*	NN	PAF
Leptomelanconium allescheri	0,497	0,04*	NN	PAF
Dermateaceae sp.	0,469	0,04*	NN	PAF
Tubaria sp.	0,473	0,016*	NN	SSF
Peethambara sundara	0,423	0,035*	NN	SSF
Hyphodontia quercina	0,727	0,001***	NN	WSF
Ascocoryne cylichnium	0,703	0,004**	NN	WSF
Phellinidium ferrugineofuscum	0,676	0,023*	NN	WSF
Corticium sp.	0,596	0,022*	NN	WSF

Tubulicrinis borealis	0,586	0,007**	NN	WSF
Climacocystis borealis	0,55	0,011*	NN	WSF
Vishniacozyma sp.	0,638	0,002**	NN	YEA

***Figure S2*. Precipitation plots for each site (mm of rain per day).** *This figure displays precipitation plots for each site, showcasing the daily rainfall in millimeters (mm). The clear-cut (CC) forest type is visually represented by the color orange, while the near natural (NN) forest type is depicted in turquoise. The red line indicates the transition from the first to the second sampling round.*



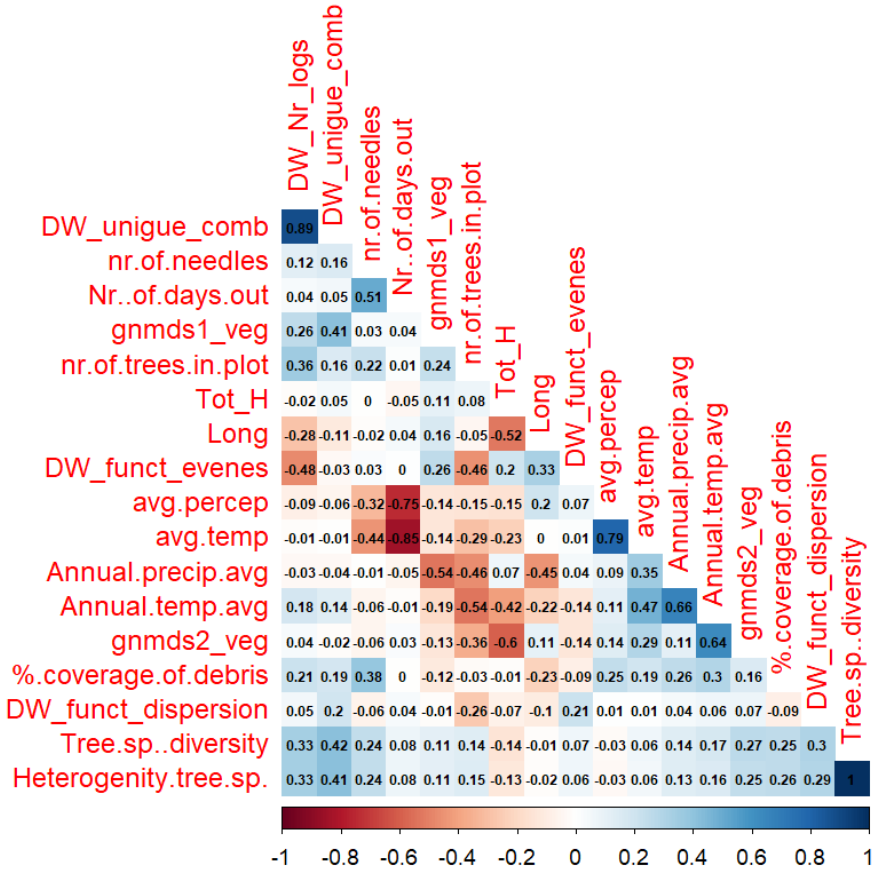


Figure S1. Correlation plot for all environmental variables. *This figure presents a correlation plot depicting the relationships among all environmental variables. The correlation values range from one to minus one, with positive correlations represented by blue and negative correlations represented by the color red. The intensity of the color gradient reflects the strength of the correlation, with darker shades indicating stronger correlations.*