



# ***Corinectria fuckeliana*: form-disturbing pathogen or agent of biodiversity?**

Long-term effects of *Corinectria fuckeliana* inoculations on stem quality and fungal communities in *Picea abies*

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*Corinectria fuckeliana*: form-disturbing pathogen or agent of biodiversity? Long-term effects of *Corinectria fuckeliana* inoculations on stem quality and fungal communities in *Picea abies*

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## Abstract

*Corinectria fuckeliana* (previously *Neonectria fuckeliana*) has for a long time been known as a weak pathogen and endophyte invading wounds of *Picea abies* and other coniferous trees. It has recently been shown to be the causal agent of cankers on *P. abies*, leading to stem deformations and top- and branch-dieback. The long-term project presented in this thesis aims to answer how these cankers shape the stem quality and fungal community in the wood. This was started in 2011, when 60 trees of *P. abies* were inoculated with wood plugs colonized by *C. fuckeliana*. The length of the lesions that developed from the inoculation were measured in 2013, 2015, and 2025. The externally measurable length of the lesion was the largest in 2015 and has since appeared to shrink. From sampled wood cores of both inoculated and non-inoculated tissue within the same tree, the increment growth was measured in three-year periods from 2025 to three years before inoculation (2008). Increment growth relative to the three years before inoculation decreased significantly over time and the decrease was initially larger in the cores sampled from inoculated wood during the first six years after inoculation, and subsequently larger in non-inoculated wood during the period 2023 - 2025. The diameter of the inoculated trees and an equally numerous group of control trees was measured at breast height (where inoculations were made), 40 cm above breast height, and 40 cm below breast height. From this, the swelling was calculated as the deviation at breast height from the average between the above and below measurements. A median swelling of one cm could be observed in the inoculated trees, and a median swelling of 0 cm could be observed in the control trees. The fungal community within the wood was analysed by extracting DNA from inoculated and non-inoculated wood from the same tree. The ITS2 region was amplified and sequenced. The inoculation treatment was able to significantly explain about 2% of the variance in the fungal communities in the wood. Shannon-Weaver diversity index, and the relative abundance of *C. fuckeliana* did not significantly differ between the treatments. The relative abundance of fungal OTUs that were classified as resinicolous did significantly differ between the treatments.

**Keywords:** *Corinectria fuckeliana*, canker, inoculation, metabarcoding, fungal community

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# Abbreviations

| <b>Abbreviation</b> | <b>Description</b>                 |
|---------------------|------------------------------------|
| ANOVA               | Analysis of variance               |
| DABH                | Diameter above breast height       |
| DBBH                | Diameter below breast height       |
| DBH                 | Diameter breast height             |
| DNA                 | Deoxyribonucleic acid              |
| IQR                 | Inter quartile range               |
| ITS                 | Internal transcribed spacer        |
| LMM                 | Linear mixed-effect model          |
| μl                  | Microliter                         |
| μm                  | Micrometer                         |
| OTU                 | Operational taxonomic unit         |
| PCR                 | Polymerase chain reaction          |
| PERMANOVA           | Permutational analysis of variance |
| RA                  | Relative abundance                 |
| SH                  | Species hypothesis                 |
| SWDI                | Shannon-Weaver Diversity Index     |

# 1. Introduction

## 1.1 Swedish forestry

Forests in Sweden are heavily influenced by industrial forestry. About 68% of Swedish land is considered forest land (SLU 2025), and 75% of Swedish forests are being actively managed for timber production. Of this forest land, 1% is harvested each year for paper pulp, timber, biofuel and energy production which in 2024 generated around SEK 185 billion in export value (Skogsindustrierna 2026). Swedish forests are dominated by the two tree species *Pinus sylvestris* L. (Scots pine) and *Picea abies* L. Karst (Norway spruce) which across all Swedish forests make up 40% and 38% of the standing volume respectively (SLU, 2025).

## 1.2 *Picea abies*

*Picea abies*, is a coniferous evergreen and is found natively in central and northern Europe, being one of the most dominant species in the boreal forests of northern Europe. *P. abies* also has a cosmopolitan distribution outside of Europe as an introduced species in North America, East Asia, Africa, and Oceania (Caudllo et al. 2016). It is usually planted in even-aged monocultures after clear-cutting and soil scarification but can also be used in mixed stands and continuous cover forestry due to it being highly shade tolerant. The rotation period in Sweden is between 60 and 100 years (Skogskunskap 2025). Like other conifers, *P. abies* produces resin as a response to insect attacks, fungal infections, and mechanical damages (Mitchel 2021). The resin is high in phenolic compounds and acts as both a chemical anti-microbial, and as a physical barrier blocking fungi from entering wounds or spreading within the tree tissue (Rautio et al. 2012). In *P. abies*, the resin causes a dark discoloration to the otherwise light wood (Temnerud 1999).

## 1.3 Endophytic fungal communities in wood

Endophytic fungi are resident within the tissues of plants without causing apparent disease. They appear to be ubiquitous in plants across biomes and tissue types, although the community composition varies greatly between them (Stone et al. 2004). The fungal communities of different compartments of a plant, like the foliage, phloem, xylem, or root tips, will vary due to factors like temperature stability, nutrient composition, moisture, and the hosts' biological activity.

The community will also vary based on the fungi themselves. Competition between fungi can be explained in three different ways. Firstly, transitive competition implies that the competitive success of different species can be organized as a hierarchy where some species are strong competitors and others are

weak, resulting in the strong ones replacing the weak ones. Secondly, intransitive competition means that species are strong competitors against some species and weak against others, resulting in a dynamic system where no one species is the top competitor which ends up replacing the other species. Finally, competition defined by priority effects is characterized by the fact that the species which are the first to colonize a substrate will have a competitive advantage over later colonizers (Bruns 2019). Fungal communities are also shaped by what species are found nearby. Distance between samples has been shown to be a good predictor of the similarity of the fungal community in dust in student dorm rooms (Adams et al. 2013), and the dispersal of ectomycorrhizal fungi have been shown to be significantly limited by distance (Peay et al. 2012).

An endophytic fungus might swap to a saprotrophic lifestyle once the tissue they inhabit dies, causing a priority effect and giving them a head start in the early colonization of newly dead tissue. This has been suggested for foliar endophytes like *Lophodermium* spp. (Minter, Millar 1979) in pine needles, and in wood where freshly cut birch logs with an endophytic community have been shown to have a higher decomposition rate than sterilized birch logs (Cline et al. 2017). Endophytes can also be latent pathogens, colonizing living plant tissue without causing apparent disease until the plant is stressed which triggers the fungus to become a disease-causing pathogen (Schultz & Boyle 2005).

## 1.4 Forest pathogens

In a natural non-managed forest ecosystem where timber productivity is not of any concern, native forest pathogens are not necessarily something to be worried about. It can rather be seen as a part of the natural disturbance cycle contributing to turnover of species in late succession forests and creating deadwood for insects and fungal decomposers, thus playing an important role for the biodiversity of the forest (Fodor & Hâruta 2022). In managed forests however, forest pathogens are a leading cause of loss of value in forestry (McTaggart et al. 2023).

A forest pathogen can be defined as a microorganism causing disease on forest trees. In turn, forest disease can be defined as abnormal functioning of a forest tree caused by a pathogen. For a disease to appear, three criteria need to be met:

1. There must be a virulent pathogen that can infect the host.
2. There must be a host which is susceptible to the pathogen.
3. There must be an environment conducive to disease.

These three criteria form the disease triangle (figure 1), which constitutes the basis of understanding diseases (Tronsmo et al. 2020).

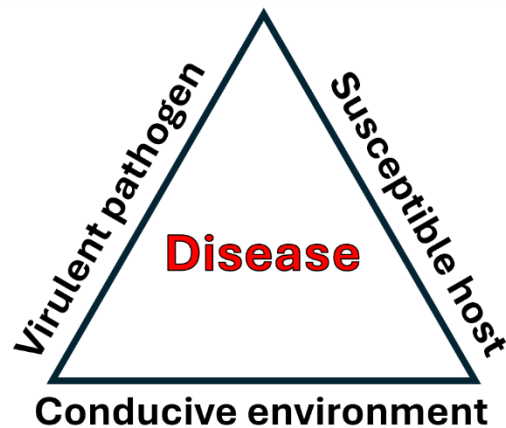


Figure 1 The disease triangle.

Fungal diseases on trees come in several forms and degrees of severity. For example, they can greatly reduce the value of the timber, like root rot caused by *Heterobasidion* spp.; they can cause systemic wilting, killing the tree within one or a few years, like the Dutch elm disease caused by *Ophiostoma ulmi* and *O. novo-ulmi*; or they can be weak pathogens, occasionally causing growth-reducing or form-disturbing cankers, like the focus organism of the work presented in this thesis, *Corinectria fuckeliana* (C. Booth) C. Gonzáles & P. Chaverri, 2017 (previously *Neonectria fuckeliana*).

In the context of forest pathology, a canker is a necrosis in the phloem and cambium region under the bark of a tree (Forestpathology 2026). Lesion is a general term for damaged or abnormal tissue caused by injury, infection or disease. A wound is an external damage to the stem where the bark has been removed and wood has been exposed.

## 1.5 *Corinectria fuckeliana*

*Corinectria fuckeliana* is an ascomycete in the family *Nectriaceae* native to the Northern hemisphere. It has been known to act as a wound invader and occasional weak pathogen of *P. abies* (Roll-Hansen & Roll-Hansen 1980; Vasilias et al. 1996), entering the wood as an endophyte through branch stumps and bark peelings. It has also been found in other coniferous trees in the genera *Abies* (Mihál et al. 2014), *Pinus* (Dick & Crane 2009) and *Larix* (Langrell 2005).

In recent years, *C. fuckeliana* has been suggested to be the causal agent of cankers on *P. abies* in Sweden and Finland (Lilja et al. 2012; Pettersson et al. 2016; Uimari et al. 2018). The ability for the fungus to be pathogenic on *P. abies* has been confirmed in inoculation trials (Pettersson et al. 2018). The cankers produced after infection by *C. fuckeliana* are characterized by necrotic phloem,

resin flow, and depressions extending above and below the cankers called flutes. From under the bark near the cankers, an erumpent stroma producing multi-septate macroconidia may appear. In this stroma, red, perithecial sporocarps around 300 – 400  $\mu\text{m}$  in diameter can appear in clusters of 10 – 100 (figure 2a). In the perithecia, several asci with eight ascospores in each are produced. When grown in pure culture on nutrient agar, *C. fuckeliana* produces orange, acremonium-like mycelium (figure 2b) with abundant conidiophores that produce hyaline and unicellular microconidia. (Booth 1979; Pettersson 2016; Gonzalez, Chaverri 2017).

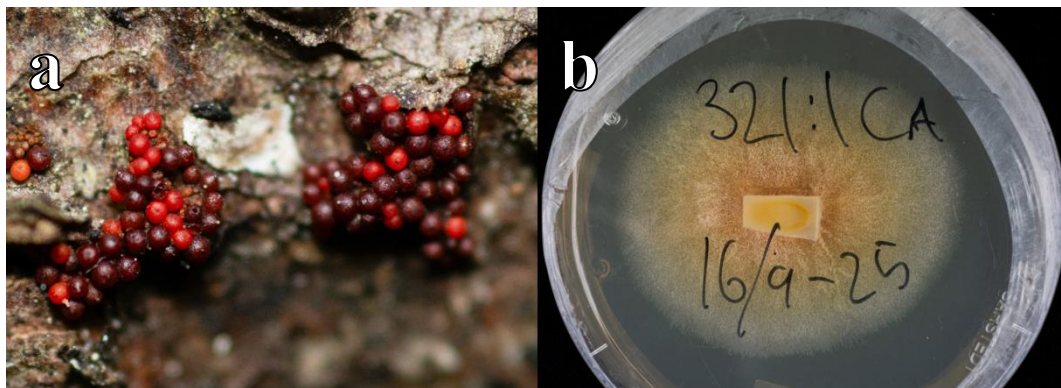


Figure 2 **a.** Perithecia produced by *C. fuckeliana* on bark of *P. abies*. **b.** Orange, acremonium-like mycelium of *C. fuckeliana* grown in pure culture on agar nutrient medium.

Presence of *C. fuckeliana* has been shown to positively correlate with attacks from the moth *Cydia pactolana* (Uimari et al. 2018) and the bark beetle *Dendroctonus micans* (Vasiliauskas et al. 1996). Whether these species act as vectors for the fungus, if they are attracted to trees infected with *C. fuckeliana*, or if their relationship is spurious and influenced by a confounding variable, remains unknown.

In Chile and New Zealand, *C. fuckeliana* has been causing disease in plantations of *Pinus radiata* as an invasive species (Gonzalez, Chaverri 2017; Dick & Crane 2009). Looking at this pathosystem from the perspective of the disease triangle, it can be said that the virulent pathogen (*C. fuckeliana*) found a susceptible host (*P. radiata*), and the humid, temperate environments of Chile and New Zealand were conducive for this disease to develop. It is not certain that *P. radiata* would develop the disease in the hot, and dry coastal environment of Southern California where it is native (Rogers 2004).

In Sweden, Christmas tree plantations of *P. abies* have been especially threatened by *C. fuckeliana* (Pettersson et al. 2018). Christmas trees are young and can thus be more easily girdled and killed. They are sometimes pruned, which causes wounds. Pruning wounds on *P. abies* have been shown to act as infection pathways for *C. fuckeliana* (Metzler 1997). The economic value of Christmas

trees comes from their aesthetic properties which could also be impaired by *Corinectria* cankers.

## 1.6 Molecular methods used in fungal community research

Polymerase chain reaction (PCR) is a method of multiplying a specific region of the deoxyribonucleic acid (DNA) molecule. It uses a primer-pair to define the start and end point of the region of interest. For example, for the study of fungal species in an environmental sample (fungal metabarcoding), the second internal transcribed spacer (ITS2) region of the ribosomal gene can be used (figure 3) (Blaalid et al. 2013). This region is non-coding, leading to it accumulating mutations without causing detrimental effects, making it highly variable between species. It is flanked by two coding regions, 5.8S ribosomal RNA and 28S ribosomal RNA, which are more conserved between species, making them suitable as primer binding sites with limited species bias. The primers used in PCR can be tagged with a unique sequence for each sample. This way, the amplicon from different samples can be pooled to a library and sequenced together. The different samples can then be separated bioinformatically.



Figure 3 Schematic illustration of the ribosomal gene in fungi. Forward primer gITS7, and reverse primers ITS4/ITS4a, and TW13 are shown as arrows. Length of regions and primer arrows roughly estimate number of base-pairs. 28S is truncated at 800 base-pairs.

## 1.7 Research questions and hypotheses

The objective of this thesis is to determine the long-term effects of the pathogenic fungus *C. fuckeliana* on *P. abies* by analyzing its local effect on the wood 14 years after inoculation. The work presented in this thesis is aimed at reaching a better understanding of the following research questions:

1. Is *C. fuckeliana* able to persist in the wood of *P. abies* several years after inoculation?
2. What long-term effects does infection by *C. fuckeliana* have on tree growth and stem quality?
3. What local effect does infection by *C. fuckeliana* have on the fungal community in the wood of *P. abies*?

It was hypothesized that:

1. *C. fuckeliana* is able to persist in the wood of *P. abies* several years after inoculation.
2. *C. fuckeliana* has a significant local effect on tree growth and stem quality due to reduced growth, fluting and swelling around the inoculation point.
3. Infection by *C. fuckeliana* shapes the fungal community in the lesions by creating a resinous environment which will benefit resinicolous fungi. *C. fuckeliana* will have a dominant presence which will reduce alpha-diversity.

## 2. Materials and method

### 2.1 Experiment background

In 2011, 60 *P. abies* trees were inoculated at four points around the stem at breast height. Three of the inoculations were done with wood plugs colonized by mycelium of *C. fuckeliana*, while the fourth was inoculated with a sterile wood plug and used as control. The information about the number of isolates used in inoculation was poorly documented. It was only stated that “[...] all trees will be inoculated with the two same isolates”. How these two isolates are distributed among the three inoculation points was not further explained. Although it was unclear whether one, two, three or even more isolates were used for inoculation, the work done in this thesis has assumed that two isolates were used and that for each inoculation, one of the two was randomly chosen.

The trees were located in Håbo, Uppsala, Sweden, and were a part of a field trial of breeding material which consisted of 74 families from 51 different parents. Every family had eight clones that were replicated three times each. There were no clonal replicates within the 60 trees selected for inoculation (appendix 1). The trees were planted in 1991 in an agricultural field in a grid with a spacing of  $1.5 \times 1.5$  meters. The site was divided into 44 parcels á  $5 \times 10$  trees. The inoculated trees were distributed in the eight parcels that make up the southwestern edge of the stand (appendix 2). The height of the trees was measured in 1997 and 2004, the diameter at the breast height (DBH) (ca 1.3 m above ground) was measured in 2004 and 2016, and the vertical length of the lesions caused by the inoculation was measured in 2013 and 2015 from the center of the inoculation to the top of the lesion.

Two of the inoculated trees were too small to get any meaningful separation of the inoculated and non-inoculated treatment and were excluded from all analysis, leaving 58 inoculated trees that were sampled and measured for the analysis presented in this thesis.

### 2.2 External measurements

#### 2.2.1 Lesion size

In 2025, the lesions' vertical length was measured in the same way as in 2013 and 2015, i.e. with a measuring tape from the inoculation point to the top of the lesion. The inoculation point was usually visible on at least one of the four inoculation points. If an inoculation point could not be found, the control inoculation (which was always visible) was used as a reference and the length was measured from that height.

The lesion length measurement from 2025 was compared with the measurements from 2013 and 2015. An analysis of variance (ANOVA) and Tukey's HSD test was performed to assess statistical difference in mean lesion length between the three timepoints.

### 2.2.2 Diameter and swelling

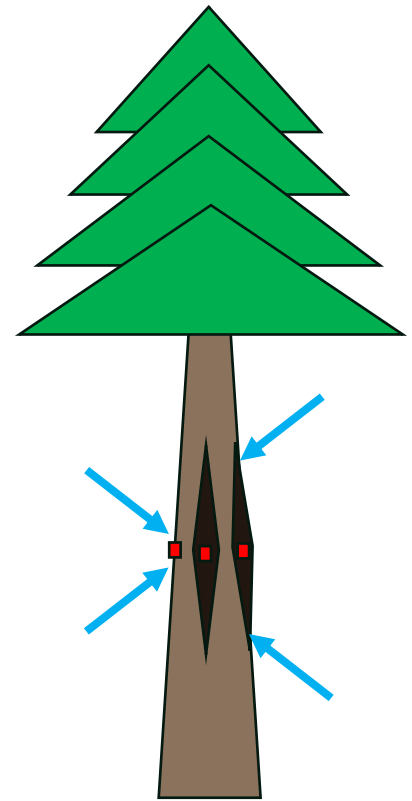
A group of control trees was selected from the stand. For each inoculated tree, a clonal or full sibling non-inoculated tree was selected for the control group, making the size of the two groups identical, and the genetic composition of the two groups as similar as possible. One tree was accidentally excluded from the inoculated group leaving the total tree count in each group at 57 trees.

The diameter of all trees in both groups was measured using calipers and rounded to the nearest half cm. Each tree was measured thrice: Once at the breast height where inoculations were made, once 40 cm above breast height (DABH), and once 40 cm below breast height (DBBH). The three measurements were used to calculate the swelling of the trunk caused by inoculation. The swelling was calculated in absolute cm for each inoculated and control tree using the following equation:

$$Swelling = DBH - \frac{DBBH + DABH}{2}$$

### 2.3 Sampling of cores

Sampling was done by taking wood cores using an increment borer with a five mm inner diameter (Haglöf® Sweden AB, Mora, Sweden). The borer was cleaned using 70% ethanol between each taken core. Four cores were taken from each inoculated tree (figure 4). Two were taken from the side inoculated with sterile wood plugs (non-inoculated), and two from the canker on the opposite side of the tree (inoculated). Each treatment was sampled once above the inoculation point and once below. The non-inoculated cores were taken two cm from the inoculation point, and the inoculated cores were taken inside the wound two cm from the edge of the wound. From the two cores taken of each treatment, one was randomly chosen for measurement of local growth and re-isolation of fungi, and the other for DNA-extraction and analysis of fungal community.



*Figure 4 Schematic illustration of inoculated tree seen from the side. Red squares represent inoculation points, dark regions on stem represent wounds, and blue arrows point to sampling points. Left side of the illustration is inoculated with sterile wood plug.*

## 2.4 Relative growth

The cores were photographed together with a piece of graph paper placed on the same elevation as the core. Each photo was taken from the same distance, with the same light source, and the same camera settings. The images were uploaded to ImageJ (Schneider et al. 2012), and the graph paper was used to set the scale. The increment growth of three-year periods was measured from the three years before inoculation to the year of sampling (2008 – 2010, 2011 – 2013, 2014 – 2016, 2017 – 2019, 2020 – 2022 and 2023 – 2025). The relative growth of each time-period was calculated by dividing the growth in that time-period with the growth during the three years before inoculation (2008 – 2010). This was done for both treatments, meaning the relative growth of each tree was measured both on a core taken from the inoculated side of the tree, and on a core taken from the non-inoculated side.

A linear mixed-effect model (LMM) was fitted to see the effect of treatment, time, and treatment  $\times$  time interaction on relative growth. Tree ID and the position of the sample (above or below inoculation point) were used as random effects. Treatment and time were used as fixed effects. For the treatment parameter, the non-inoculated treatment was set as the baseline. For the time parameter, the year-range 2008-2010 was set as the baseline. An ANOVA was fitted on the model to test if the model parameters (treatment, time and treatment  $\times$  time) were different from zero.

## 2.5 Dark pixels in cores

To estimate resin content in the wood, the percentage of pixels in the photographs of the cores that were darker than an auto threshold was calculated in ImageJ. A polygon selection was made of the core up to the center of the tree. The image type was changed to 8-bit and an auto threshold was set using the *IsoData* method (figure 5). The *Analyze Particles* function was used to measure the area of the pixels darker than the threshold. Differences in the percentage of dark pixels

in the selected area in the photos were tested using a Mann-Whitney U-test due to non-normal distributions (appendix 3).

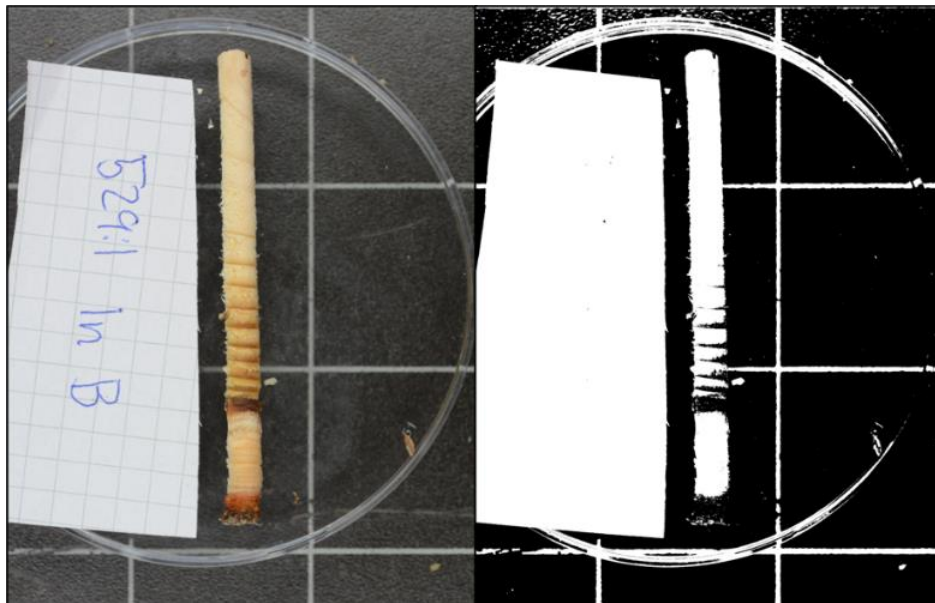


Figure 5 Image analysis of an inoculated wood core. Left image shows unedited photo; right image displays all the pixels darker than the IsoData auto threshold as black.

## 2.6 Re-isolation and somatic incompatibility

After photographing, the cores were surface sterilized by passing them quickly through a flame and placed on Hagem media agar (Stenlid 1985). Over the following days, the cores were monitored and any mycelium growing out from the cores that showed signs of the orange, acremonium-like morphology of *Corinectria* spp. (figure 2b) was cut out and placed on new plates.

In an attempt to understand if the isolates were the same genet (and thus likely one of the two isolates used in inoculation), a small trial of somatic incompatibility testing was set up. Somatic incompatibility trials work by assuming fungal mycelia can recognize self from non-self and fuse with the prior while creating an incompatibility zone against the latter (Boddy 2015). The trial was set up by selecting 68 isolates, dividing them into groups of four, and pairing every isolate against each other within the groups, creating 102 pairs. Of the 68 isolates selected for the trial, 10 isolates were randomly chosen to be self-paired and used as a control. Two 5 mm circular discs of edge mycelia were placed 2 cm apart on plates of agar vegetable media (Vasiliauskas & Stenlid 2011). The trial was observed after 60 days, if the two mycelia had fused, they were considered one genet. If they had created an incompatibility zone, they were considered two separate genets.

## 2.7 DNA extraction for fungal community analysis

To prepare the wood cores for DNA extraction, they were freeze dried using a ScanVac CoolSafe® (LaboGene, Allerød, Denmark) for 3 days. The outer bark was removed before a MM400® ball mill (Retsch, Haan, Germany) was used with 10 ml steel chambers and two 10 mm steel balls per chamber to homogenize the dry cores. DNA was extracted using the Nucleospin® soil kit (MACHEREY-NAGEL, Düren, Germany). For each sample, 75 mg of the homogenized wood was placed in a 2 ml screwcap tube with two M6M M3 8 FZB bolt nuts (Botnia Bolt®, Kristiinankaupunki, Finland), and mixed with 1200 µl of SL1 buffer at 5500 rpm for 2x30 seconds with a 10 second pause using a Precellys® 24 touch homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France). 150 µl of SL3 buffer was added and incubated on ice for 5 minutes. Lysates were filtered using the Nucleospin® Inhibitor Removal Ring after which 250 µl of SB buffer was added. The sample was loaded onto the Nucleospin® Soil Column, which was then washed with 500 µl SB buffer, 550 µl of SW1 buffer, and 2 x 700 µl of SW2 buffer. The column was dried by centrifuging, and DNA was eluted using 50 µl of SE buffer. The extraction was done in batches of 24 samples, and each batch included a negative control which followed all the same steps without adding the 75 mg of wood.

After extraction, the DNA concentration was measured using a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and diluted to a concentration of 1 ng/µl. 25 µl of diluted DNA was mixed with 8.25 µl of pure water from a PURELAB® Chorus 2 (ELGA LabWater (VWS Ltd.) High Wycombe, UK), 5 µl of 10x DreamTaq® Green Buffer (Thermo Fisher Scientific, Vilnius, Lithuania), 5 µl of dNTP, 1.5 µl MgCl<sub>2</sub>, 0.25 µl of Dreamtaq® DNA Polymerase, and 5 µl of tagged gITS7 (5'TGARTCATCGARTCTTTG) and TW13 (3'GCAGAACTTTGTGCCTGG) primer mix (figure 3) (Ihrmark 2012), resulting in 50 µl of PCR product. To limit contamination, the mixing of PCR ingredients was done in a UV-hood with filter pipette tips. The PCR program (table 1) was first run for 25 cycles, after which the amplicon was tested using electrophoreses. Samples which did not get sufficient amplification due to inhibition or low concentration of fungal DNA were rerun in a new PCR. First for 28 cycles, and then 35 if needed. Samples that could not be amplified to sufficient levels this way were rerun again using Terra® polymerase and buffer (Takara Bio USA, Inc., Mountain View, CA, US). These were run with 3 µl of undiluted DNA extract, 8.4 µl of pure water, 15 µl Terra® Direct PCR Buffer, 0.6 µl Terra® Direct PCR Polymerase Mix, and 3 µl of the same primer pair mix, resulting in 30 µl of PCR product. The PCR program was run for 25 cycles. Each PCR run included a negative control which was mixed with the same reagents in the same hood but with the 25 µl of DNA sample replaced with pure water. The negative controls were checked for bands in the electrophoreses.

*Table 1 Time and temperatures used for the DreamTaq and Terra enzyme in denaturing-, annealing-, and elongation phase in the PCR reaction.*

| <b>Enzyme</b> | <b>Denaturing</b> |                 | <b>Annealing</b> |                 | <b>Elongation</b> |                 | <b>Cycles</b> |
|---------------|-------------------|-----------------|------------------|-----------------|-------------------|-----------------|---------------|
|               | <b>Time(s)</b>    | <b>Temp(C°)</b> | <b>Time(s)</b>   | <b>Temp(C°)</b> | <b>Time(s)</b>    | <b>Temp(C°)</b> |               |
| DreamTaq      | 30                | 95              | 30               | 57              | 60                | 72              | 25, 28, 35    |
| Terra         | 30                | 98              | 30               | 56              | 90                | 68              | 25            |

After amplification, the samples were cleaned using AMPure XP® beads (Beckman Coulter, Inc., Brea, CA, USA). Their concentrations were measured using a Qubit® Flex Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). From these measurements 40 ng of amplified DNA was taken from each sample and pooled into two pools. One PCR-control was included in each pool that was sent for sequencing. The volume of PCR product from the negative controls added to the pools was calculated as the mean value added from all the other samples. The pools were diluted 1 part sample to 5 parts elution buffer. A final cleaning was done using E.Z.N.A® Cycle Pure Kit (Omega Bio-Tek, Inc., Norcross, GA). Sequencing was done by SciLifeLab using the PacBio Revio® platform (Pacific Biosciences, Menlo Park, CA, USA).

## 2.8 Data processing and statistical analysis

All statistical analyses were done in R version 4.4.2 in R-studio and Windsurf. Coding was assisted by Claude Sonnet 4.5. The dplyr (Wickham et al. 2023) package was used for general data management. All plots were created using the ggplot2 (Wickham 2016) and patchwork (Pedersen 2025) packages. Box plots are plotted using standard settings (line at median, box at second and third quartile, whiskers at 1.5 \* second and third quartile, and datapoints outside of whiskers shown as points). Tables were generated using the knitr (Xie 2025) and kableExtra (Zhu 2024) packages. Normal distributions were tested and visualized using Shapiro test and QQ-plots.

### 2.8.1 Managing metabarcoding dataset

The raw PacBio sequence data was processed through the SCATA pipeline (Durling 2011, <https://scata.mykopat.slu.se/>). For the 5' primer sequence, the same gITS7 primer that was used in amplification was used and the proportional primer match was set to 0.9. For 3' primer sequence, ITS4/ITS4a (3'CGTATAGTTATTCSCTCC) was used at 0.8 proportional primer match. The primer match was set lower for ITS4/ITS4a since this region was sequenced through rather than amplified at the primer bind site, leaving it with a larger variability. This primer pair is roughly a third of the length of the gITS7 - TW13 pair used in sequencing. Since the longer primer pair contains a larger proportion of conserved base pairs, using it for clustering could result in different species

being clustered together. Genotypes with only one read were removed in SCATA. The output file from SCATA was a table where each row was a tagged sample, and each column was an operational taxonomic unit (OTU). The number in each cell was the read count of the specific OTU with the specific tag. The tags were replaced with the tree ID and treatment of that sample. Full parameter set used for clustering in SCATA can be found in appendix 4.

All OTUs were run through UNITE SH Matching (v2.0.0, unprocessed) (Bengtsson-Palme 2013) on the PlutoF platform to identify and remove non-fungal OTUs. After removal of non-fungal OTUs, the remaining fungal sequences were blasted using NCBI nBlast tool (Sayers et al. 2025) with the Core nucleotide database. The 75 most abundant OTUs were manually given a species hypothesis (SH) based on the nBlast results. The highest taxonomic level available with the highest percent identity match was chosen. If a species with less than 97.5% match was found, the OTU was given a lower taxonomic level SH.

To identify and remove potential contaminations, the data set was scanned for OTUs that consistently appeared in the control samples and with similar read counts in the rest of the samples. One such OTU, blasted as *Malassezia restricta*, was identified and removed from the analysis. To further improve statistical analysis, OTUs occurring in two or fewer samples were removed (this step was skipped for data used for measurements of alpha diversity). Relative abundance (RA) of each OTU was calculated by dividing the read count of each sample with the total read count of all OTUs within the sample.

### 2.8.2 Alpha diversity

Shannon-Weaver diversity index (SWDI) was used as the measurement of alpha-diversity. A dataset of absolute abundances including the OTUs occurring two or fewer times was run through the diversity function in the vegan package (Oksanen et al. 2025) with Shannon selected as index. The median SWDI was compared between the treatments using the Mann-Whitney U-test due to non-normal distribution (appendix 3).

### 2.8.3 Non-metric Multi-Dimensional Scaling (NMDS)

A two-dimensional NMDS was created with the metaMDS function using Bray-Curtis dissimilarity index, 100 random starts, and a seed of 123. A permutational analysis of variance (PERMANOVA) was run on the NMDS scores using the adonis2 function with Bray-Curtis dissimilarity index, 999 permutations and a seed of 123, testing for grouping by treatment (inoculated and non-inoculated). Another NMDS and PERMANOVA were run with the same settings and parameters but instead grouping by polymerase enzyme used in PCR (DreamTaq® or Terra®) to test for bias in the amplification. The NMDSs were

plotted using the ggplot2 package and ellipses were drawn using the type “norm”. Both the metaMDS and adonis2 functions are from the vegan package.

#### 2.8.4 Effect of distance on fungal community

Distance between trees was tested as an explanatory variable for fungal community dissimilarity. Two matrices were created with every tree-tree pair, resulting in 1596 pairs. In one matrix, the distance between each tree-pair was calculated using the Pythagorean theorem, assuming all trees are planted in a perfect grid with a spacing of  $1.5 \times 1.5$  meter (appendix 2). For the other matrix, the fungal community of the two treatments was pooled for each tree, and the read counts were transformed to RA. Each tree pair was compared using the vegdist function from the vegan package with Bray-Curtis dissimilarity index. The two matrices were combined and converted to a long format so that every tree-pair got a distance value and a dissimilarity value. A linear regression model was fitted using the base R lm function and the data was plotted as a scatter plot together with the linear model using the ggplot2 package.

#### 2.8.5 Treatment effect on relative abundances

Among the 75 OTUs that were given an SH, resinicolous fungi were identified using the list of fungi in the “Resinicolous Fungi” project on INaturalist (INaturalist 2026), along with specific searches in Google Scholar for “<Species name> AND resinicolous”. The RAs of OTUs that were identified as resinicolous were pooled to get the total RA of resinicolous fungi. Due to a non-normal distribution (appendix 3), the difference in RA of both resinicolous fungi and *C. fuckeliana* was compared between treatments using a Mann-Whitney U-test.

### 3. Results

#### 3.1 Long-term development of lesions

The mean vertical length of all lesions was observed to be the largest in 2015 at 39.8 cm and the smallest in 2013 at 9.1 cm. In 2025 the mean length was back down to 13.2 cm, which is still significantly higher than in 2013 according to ANOVA and Tukey's HSD test at  $p < 0.05$  (figure 6a).

The ANOVA run on the LMM revealed significant effects of the model parameters: treatment ( $p = 0.001$ ), time ( $p < 0.001$ ), and treatment  $\times$  time ( $p < 0.001$ ) (table 2). Both treatments showed a decline in relative growth over time (all time periods  $p < 0.001$ ). The treatment  $\times$  time interaction revealed temporal variation in the effect of treatment on relative growth. During 2011-2013 inoculated samples showed significantly greater growth reduction than non-inoculated samples (estimate = -0.405,  $p < 0.001$ ). This difference persisted but diminished by 2014-2015 (estimate = -0.209,  $p < 0.001$ ). For the years 2017-2019, and 2020-2022, no significant treatment effect can be seen. By 2023-2025, the inoculated samples show slightly higher relative growth than non-inoculated samples (estimate = 0.112,  $p = 0.0451$ ) (figure 6b; table 3).

*Table 2 Type III ANOVA results for the linear mixed-effect model of relative growth testing the overall significance of treatment, time, and their interactions*

| Effect                  | NumDF | DenDF   | Sum Sq | Mean Sq | Fvalue | Pr(>F) |
|-------------------------|-------|---------|--------|---------|--------|--------|
| Treatment               | 1     | 364.546 | 0.464  | 0.464   | 10.46  | 0.0013 |
| Time                    | 5     | 586.230 | 22.734 | 4.547   | 102.50 | <0.001 |
| Treatment $\times$ Time | 5     | 586.230 | 6.028  | 1.206   | 27.18  | <0.001 |

*Table 3 Fixed effects estimate of the linear mixed-effect model of relative growth, showing the effects of treatment, time, and their interactions.*

| Parameter                        | Estimate | Std. Error | t-value | Pr(> t ) |
|----------------------------------|----------|------------|---------|----------|
| Intercept (Non-inoc., 2008-2010) | 1.002    | 0.034      | 29.53   | <0.001   |
| Inoculated effect (2008-2010)    | -0.002   | 0.041      | -0.06   | 0.953    |
| Time: 2011-2013                  | -0.126   | 0.039      | -3.22   | 0.00134  |
| Time: 2014-2016                  | -0.202   | 0.039      | -5.17   | <0.001   |
| Time: 2017-2019                  | -0.539   | 0.039      | -13.77  | <0.001   |
| Time: 2020-2022                  | -0.559   | 0.039      | -14.28  | <0.001   |
| Time: 2023-2025                  | -0.559   | 0.039      | -14.29  | <0.001   |
| Inoc. $\times$ 2011-2013         | -0.405   | 0.056      | -7.27   | <0.001   |
| Inoc. $\times$ 2014-2016         | -0.209   | 0.056      | -3.75   | <0.001   |
| Inoc. $\times$ 2017-2019         | 0.072    | 0.056      | 1.29    | 0.198    |
| Inoc. $\times$ 2020-2022         | 0.079    | 0.056      | 1.42    | 0.157    |
| Inoc. $\times$ 2023-2025         | 0.112    | 0.056      | 2.01    | 0.0451   |

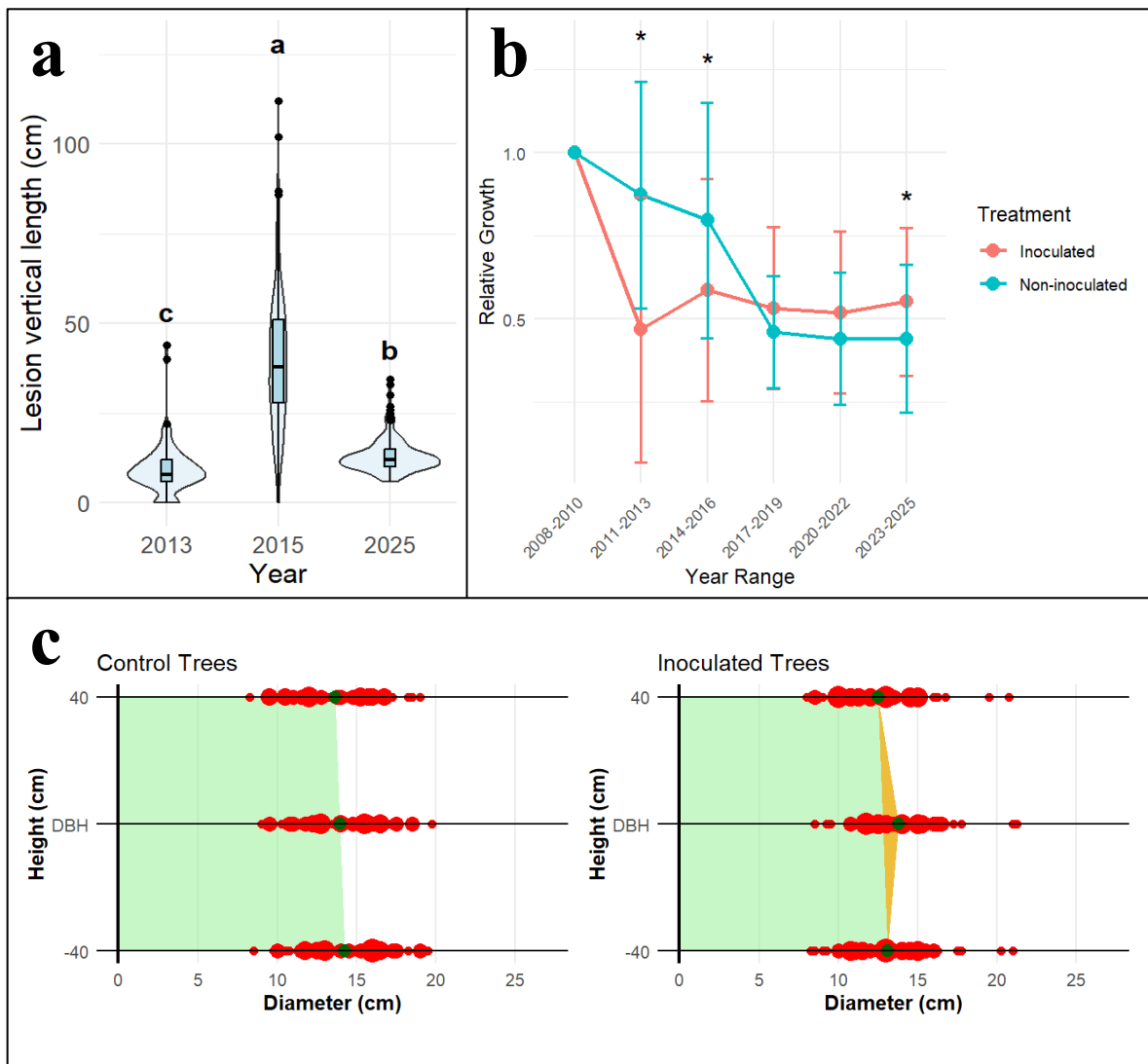


Figure 6 **a**. Mean lesion length by year measured externally from inoculation point to top of lesion. Results from ANOVA and following Tukey HSD are shown as letters above plots. Means with different letters are significantly different at  $p < 0.05$  **b**. Growth in three-year periods from 2011-2025 relative to the growth during the three years prior to inoculation (2008-2010). Asterisks denote significant treatment  $\times$  time interaction terms ( $p < 0.05$ ), indicating that the estimated gap between treatments at those time points differed significantly from the estimated gap at baseline (2008-2010) (table 3) **c**. Diameter of inoculated and control trees measured at breast height, 40 cm above breast height, and 40 cm below breast height. The mean diameter at each height is displayed with a dark green point. The mean swelling is displayed as an orange triangle.

The median swelling size at breast height, calculated from the diameter measurements (figure 6c) was significantly different between the two treatments at  $p < 0.001$  (table 4). The median swelling of inoculated trees was 1 cm with an interquartile range (IQR) of 0.625 cm, while the median of non-inoculated trees was 0 cm and an IQR of 0.375.

Table 4 Results of Mann-Whitney U-test comparing the medians of the percentage of dark pixels in photographs of cores, RA of *C. fuckeliana*, RA of resinicolous fungi, SWDI, and Swelling at breast height of inoculated trees and control trees.

| Variable        | Inoculated Cores |        |    | Non-inoculated Cores |       |    | Test Statistics       |       |         |
|-----------------|------------------|--------|----|----------------------|-------|----|-----------------------|-------|---------|
|                 | Median           | IQR    | n  | Median               | IQR   | n  | U                     | r     | p-value |
| %Dark pixels    | 12.912           | 14.141 | 58 | 9.296                | 7.154 | 58 | 2043                  | 0.185 | 0.047 * |
| RAC. fuckeliana | 0.077            | 0.142  | 57 | 0.067                | 0.157 | 57 | 1829                  | 0.109 | 0.248   |
| RAResinicolous  | 0.161            | 0.208  | 57 | 0.119                | 0.178 | 57 | 1982                  | 0.190 | 0.043 * |
| SWDI            | 2.635            | 0.674  | 57 | 2.693                | 0.850 | 57 | 1517                  | 0.057 | 0.544   |
| Swelling        | Inoculated Trees |        |    | Control Trees        |       |    | 3128.5 0.801 <0.001 * |       |         |
|                 | 1.000            | 0.625  | 57 | 0.000                | 0.375 | 57 |                       |       |         |

## 3.2 Dark pixels

The percentage of the pixels in the photos of the cores which was darker than the *IsoData* auto threshold differed significantly between the two treatments ( $p = 0.047$ ) (figure 7; table 4). The median coverage of dark pixels in inoculated cores was 12.9% with an IQR of 14.1 while the median coverage in non-inoculated cores was 9.3% with an IQR of 7.2.

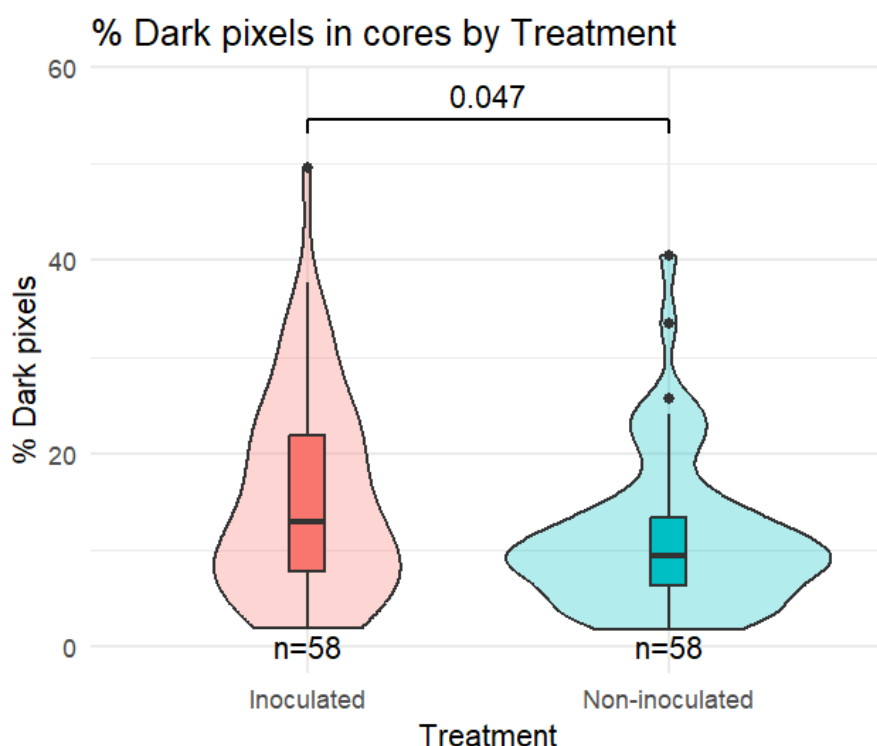


Figure 7 Boxplot and violin plot of the percentage of Dark pixels in cores by treatment. P-value from Mann-Whitney U-test is shown above the plots.

### 3.3 Re-isolation and somatic incompatibility of *Corinectria* isolates

Isolation of mycelia that were morphologically identified as *C. fuckeliana* was successful from all but 3 of the trees. One of the trees only had successful re-isolation from the non-inoculated sample, and 22 of the trees only had successful re-isolations from the inoculated samples. 32 trees had successful re-isolations from both samples (table 5).

Table 5 Number of trees where isolation of *Corinectria* mycelium was successful in only the inoculated sample, only non-inoculated sample, both samples, and none of the samples.

| Sample type                  | Number of trees |
|------------------------------|-----------------|
| Only inoculated sample       | 22              |
| Only non-inoculated sample   | 1               |
| Both samples                 | 32              |
| None of the samples          | 3               |
| <b>Total number of trees</b> | <b>58</b>       |

Clearly defined somatic incompatibility was only found in one of the 102 pairings in the trial and neither of the isolates in the incompatible pair was incompatible with any other isolates. The trial was considered inconclusive and unreliable for predicting if the isolates were different genets, so no full-scale somatic incompatibility trial was pursued.

### 3.4 Fungal community in wood

Of the 58 trees sampled, extraction and amplification was successful for 57 of them. One inoculated sample was accidentally removed during pooling of the PCR-products, and the non-inoculated sample from the same tree was removed from the analysis to keep the dataset balanced, resulting in 114 samples.

The raw data from the sequencing contained 11,265,675 reads. Out of these, roughly 69% passed the SCATA quality filtering resulting in 7,800,674 reads being used for clustering. After filtering away plant DNA, contamination, and control samples, the total read count was down to 314,247, meaning only about 2.8% of the total reads were used in the analyses.

The clustering from SCATA resulted in 1,893 OTUs. After removing OTUs belonging to plants and contamination, 701 fungal OTUs remained (represented by 314,247 reads). Out of these, 380 occurred in only two of the samples and were removed from all statistical analyses except for SWDI, leaving 321 fungal OTUs for the rest of the analyses.

The PERMANOVA analysis of the polymerase enzyme used in PCR showed no significant effect of enzyme on the fungal community (appendix 5).

The PERMANOVA analysis of the treatment showed that less than 2% ( $R^2 = 0.019$ ) of the variation observed in fungal communities can be significantly explained by the treatment variable ( $p = 0.01$ ) (figure 8). The NMDS had a stress level of 0.282.

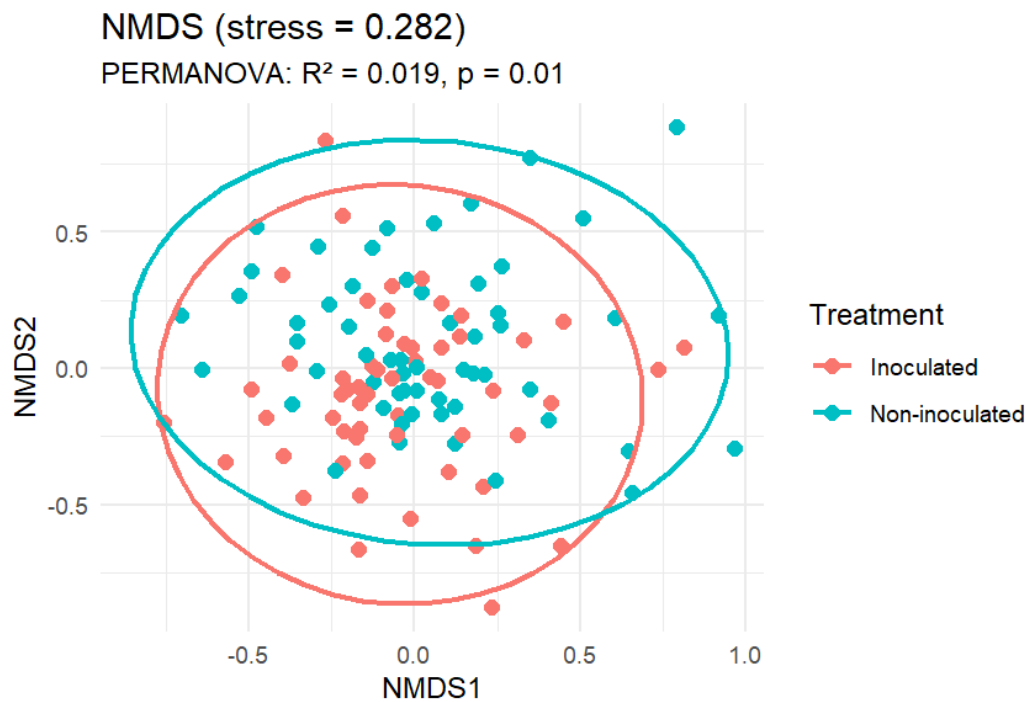


Figure 8 NMDS ordination of the samples coloured by treatment. Stress level of NMDS, along with  $R^2$  and  $p$ -value from PERMANOVA are displayed above the plot.

### 3.4.1 Distance effect on fungal community

At the spatial scale of this site, distances between trees could only significantly explain about 0.4 % of the variance in dissimilarities of the fungal communities within the trees (figure 9). The longest distance between sampled trees was 57.2 meters. Although the  $p$ -value was significant ( $p < 0.014$ ), this is likely an artifact of the large sample size that comes from pairing every inoculated tree with each other. Distance between trees was not used as a controlling variable in any of the analyses.

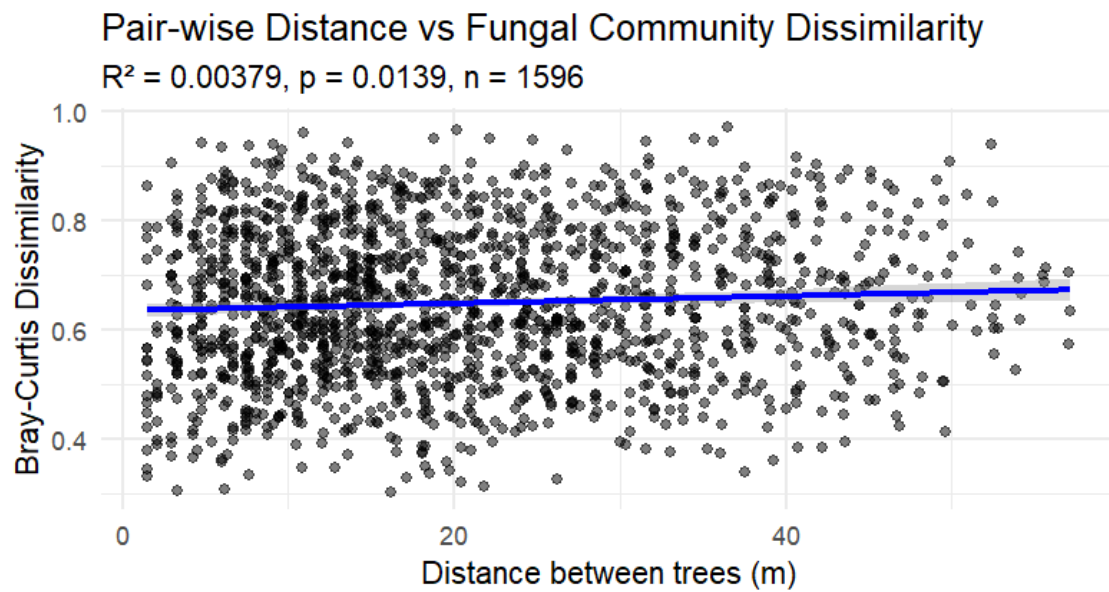


Figure 9 Pair-wise Bray-Curtis dissimilarity in fungal community composition plotted against pair-wise distance between inoculated trees in the stand. Each point represents a tree-tree pair. Self-pairings are not included. The  $R^2$  and  $p$ -values displayed above the plot are from the linear regression model.

### 3.4.2 Alpha diversity.

The Mann-Whitney U-test comparing the mean SWDI in inoculated and non-inoculated samples did not show any significant difference between the two treatments ( $p = 0.54$ ) (figure 10; table 4).

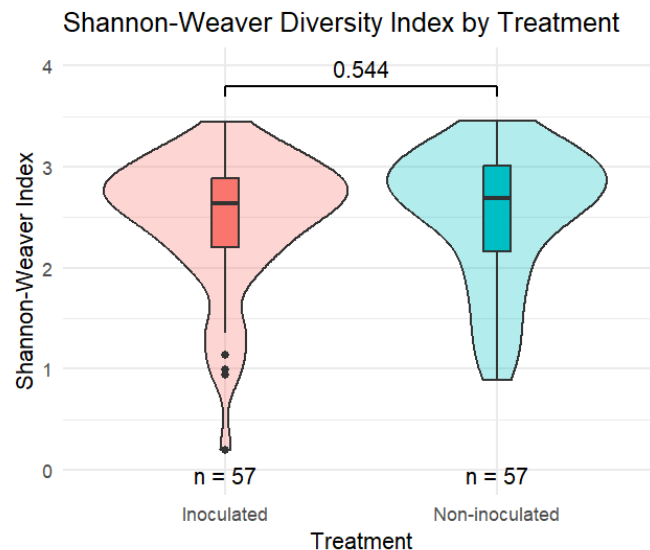


Figure 10 Boxplot and violin plot of SWDI by treatment.  $P$ -value from Mann-Whitney U-test is shown above the plots.

### 3.4.3 Species hypotheses

*Corinectria fuckeliana* was the most abundant OTU found in the samples, making up about 13.4% of all fungal reads. It was followed by *Lophium arboricola* at about 10% of all reads (appendix 6). The species *Sarea resinae* and *Sarea difformis* both occurred twice as separate OTUs. These OTUs matched with different reference sequences with the same species name (appendix 7).

Nine OTUs of seven different species were identified as resinicolous among the 75 most abundant OTUs (table 6).

Table 6 Resinicolous fungal taxa identified among the 75 most abundant OTUs. Species hypotheses were assigned based on NCBI nBLAST matches to reference sequences, with percent identity indicating sequence similarity. Sources confirm the resinicolous ecology of each species.

| Cluster nr.  | Species Hypothesis              | Reference sequence | Percent identical | Source                     |
|--------------|---------------------------------|--------------------|-------------------|----------------------------|
| scata6928_1  | <i>Lophium arboricola</i>       | OM614739.1         | 100.00            | Czachura & Janik 2024      |
| scata6928_7  | <i>Sarea difformis</i>          | MH857896.1         | 99.60             | Beimforde et al. 2020      |
| scata6928_11 | <i>Claussenomyces olivaceus</i> | KY661432.1         | 100.00            | Hawksworth & Sherwood 1981 |
| scata6928_10 | <i>Sarea resinae</i>            | MN720377.1         | 100.00            | Beimforde et al. 2020      |
| scata6928_13 | <i>Sarea difformis</i>          | MT809287.1         | 100.00            | Beimforde et al. 2020      |
| scata6928_16 | <i>Sarea resinae</i>            | MT809245.1         | 100.00            | Beimforde et al. 2020      |
| scata6928_25 | <i>Sorocybe oblongispora</i>    | MN114115.1         | 95.40             | Crous et al. 2019          |
| scata6928_59 | <i>Sarea coeloplata</i>         | MT809285.1         | 100.00            | Beimforde et al. 2020      |
| scata6928_84 | <i>Lachnellula resinaria</i>    | MT913605.1         | 98.33             | Minter 2005                |

### 3.4.4 Relative abundances

The Mann-Whitney U-test did not show any difference in the median RA of *C. fuckeliana* between the treatments ( $p = 0.248$ ) but did show a significant difference in the median RA of resinicolous fungi between treatments ( $p = 0.043$ ) (figure 11; table 4). The inoculated median RA of resinicolous fungi was 0.161 with an IQR of 0.208 and the non-inoculated median was 0.119 with an IQR of 0.178.

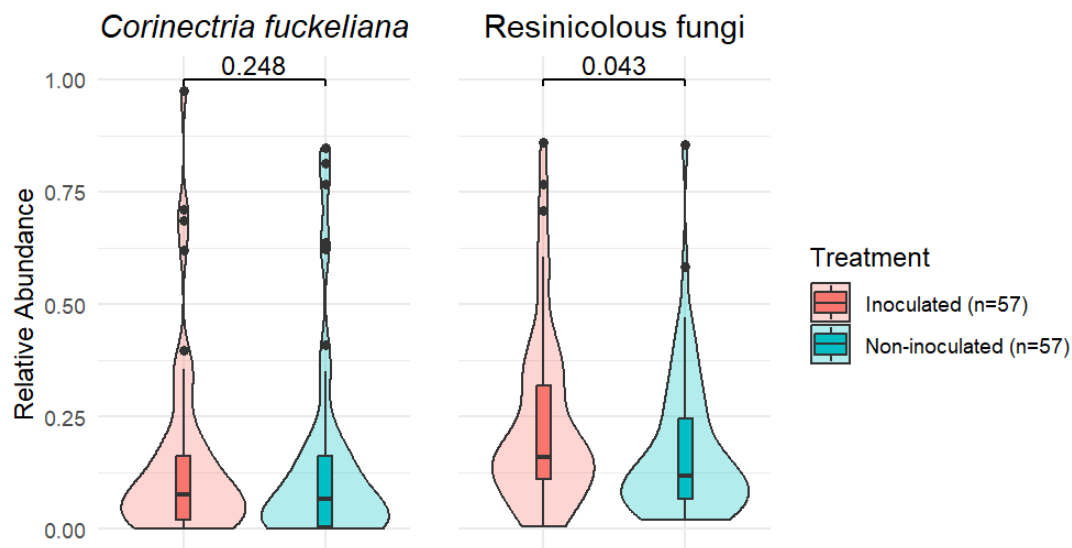


Figure 11 Violin plot and boxplot of RA of *C. fuckeliana* (left) and resinicolous fungi (right) by treatment. P-values from Mann-Whitney U-tests are shown above the plots.

## 4. Discussion

### 4.1 Methodology

#### 4.1.1 Sampling

Because of the risk of causing damage to trees intended for timber production, healthy trees in the stand were unable to be sampled with the increment borer. If it would have been possible, these samples would have been included in all the same processes as the samples that were able to be collected: relative growth measurement, isolating mycelia of *C. fuckeliana*, and metabarcoding analysis. This would have acted as a more robust negative control, complementing the sampling of the non-inoculated side of the inoculated trees.

#### 4.1.2 Identification of fungal isolates and somatic incompatibility

Identification of the isolated fungal mycelium was only done morphologically. Although mycelia of *Corinectria* spp. is characteristic and easily identifiable, it is possible that some isolates were misidentified. Mycelia of other species in the *Nectriaceae* family like *Neonectria* (Stauder et al. 2020) or *Nectria* (Hirooka et al. 2012) could be morphologically similar to that of *C. fuckeliana*. A more robust identification of the isolates could have been done by sequencing the ITS2 region of the isolates. Furthermore, sequencing several genetic markers could be done to

identify the population structure of the isolates, which the somatic incompatibility trial was unable to do.

#### 4.1.3 Primers

The general gITS7 primer was chosen over the fungal specific fITS7 primer (Ihrmark et al. 2012) as the forward primer for the PCR. This choice was made to match the method used in a similar experiment in Latvia (Daniels Loris 2023) for future comparisons of the two experiments. However, even though the same forward primer was used, results might be difficult to compare anyways. The sequencing in the Latvian experiment was made using the RS II PacBio platform, while the work presented in this thesis used the Revio PacBio platform. The different platforms could introduce taxonomic bias in the sequencing based on factors like GC-content and amplicon length. If a mock community with a known composition was sequenced on both platforms, this could potentially be used to control for sequencing bias. Nevertheless, even though a diverse fungal community could be seen from the 3% of the sequenced reads that were usable, it could have been better to use the fITS7 primer to reduce the amount of amplified plant DNA that was sequenced. No comparison between the two experiments has yet been made.

#### 4.1.4 Clustering and species hypotheses

Two species, *Sarea difformis* and *Sarea resinae*, were both represented twice in the OTU table. *Sarea* is a genus which has undergone taxonomic revisions in recent years leading to the genus splitting into *Sarea*, *Zythia*, and *Astrozythia* (Mitchell et al. 2021). *S. resinae* was moved to the genus *Zythia* (but will continue to be called *Sarea resinae* in this thesis for consistency). While *S. resinae* produces bright orange apothecial sporocarps, *Astrozythia klamathica*, *Sarea difformis*, and *Sarea coeloplata* all produce black apothecial sporocarps, meaning misidentification of the reference sequences for these species could be possible, especially before the split of the genus *Sarea*. Similarly, *S. resinae* could be a species complex that might be further divided in the future. For example, one of the OTUs (scata6928\_16), had a single 100% match with a sequence identified as *Zythia pinastri* along with several 100% matches with *S. resinae* references. For the study presented in this thesis, this has little relevance since the entire *Sarea* genus is considered resinicolous (Beimforde et al 2020).

### 4.2 RQ1: Is *C. fuckeliana* able to persist in wood of *P. abies* several years after inoculation?

The high rate of successful re-isolations of *C. fuckeliana* mycelia from the wood suggests that the fungus has persisted since inoculation. This is further

supported by the high abundance of *C. fuckeliana* DNA in the wood. The fact that *C. fuckeliana* DNA was found in equal relative abundance in both treatments (figure 11) indicates that the fungus' presence extends out of the lesion into sound looking wood, as has been shown in earlier studies (Huse 1981; Roll-Hansen & Roll-Hansen 1979). Although DNA was found at similar abundances in the two treatments, re-isolation had a higher success rate from inoculated samples than non-inoculated ones, which could suggest that the mycelium is more active and competitively viable on the side of the tree that was inoculated.

An alternative explanation to the high abundance of *C. fuckeliana* DNA and mycelia is that this does not come from the isolate(s) used in inoculation, but rather from mycelia already present prior to inoculations, or secondary colonization events after inoculation. *C. fuckeliana* is known to be one of the most common wound invaders of *P. abies*. A natural colonization of native *C. fuckeliana* would be characterized by high genetic diversity where each colonization would be from a different fungal genet (Vasiliauskas & Stenlid 1997), whereas the isolates used in inoculation were just two genets. Until a successful somatic incompatibility test is performed, or until the isolates are further sequenced to reveal the population structure of the isolates found at the site, this alternative explanation cannot be fully rejected. Comparing RA of *C. fuckeliana* from non-inoculated control trees which were unable to be sampled in this study would also help explain whether the high abundance of *C. fuckeliana* found in the wood was due to inoculation or natural presence.

### 4.3 RQ2: What long-term effects does infection by *C. fuckeliana* have on tree growth and stem quality?

The data visualized in figure 6 paints a picture of the development of the lesions caused by *C. fuckeliana* in otherwise healthy individuals of *P. abies*. At the start of infection, increment growth was greatly reduced or even halted around the lesion (figure 6b). This causes a depression to extend along the length of the trunk (figure 12a), much like the fluting seen in *P. radiata* in Chile and New Zealand (Morales 2009; Dick & Crane 2009). The flute keeps growing over a couple of years as disease symptoms get worse. As the tree attempts to defend itself, it allocates resources to the diseased area (Schultz et al. 2013), causing a local increase in growth which leads to the trunk swelling around the inoculation point. Eventually, the tree begins callousing over the lesion, and the flute becomes less prominent as healthy tissue fills the depression. Wound closing has been shown in wounds colonized by *C. fuckeliana* in previous studies (Vasiliaskas & Stenlid 1996). For an external observer, the lesions appear to have shrunk (figure 6a).



Figure 12 **a**: Photo from 2015 of an inoculated tree. A depression can be seen extending vertically from the inoculation point. **b**: Photo from 2025 of another inoculated tree. Wounds caused by inoculation of *C. fuckeliana* can be seen under blue mark. No apparent depression can be seen extending from the top or bottom of the wounds.

A more comprehensive picture of the development of the disease could have been achieved if the lesions were measured continuously every two years or so. In the ten-year time gap between 2015 and 2025, the apparent lesion length was dramatically reduced. The long time-period without measurements leaves several

questions unanswered: was 2015 the lesion-length-maximum? After lesion length started to reduce, did it reduce sharply until stabilizing on 2025-levels, or did it reduce gradually over time?

It should also be noted that the measurement of the lesion length was limited by what was externally visible. Two and four years after inoculation in 2013 and 2015, the extent of the lesion could be clearly visible as a depression extending along the stem (figure 12a), while in 2025, only the wounds and dried resin was visible as the depressions had been rounded over (figure 12b). Although the results seen in figure 6a represent a real visible effect, it is important to keep in mind that this effect does not necessarily represent an actual reduction of the lesion length but rather that the externally measurable symptoms have been concealed.

#### 4.4 RQ3: What local effect does infection by *C. fuckeliana* have on the fungal community in the wood of *P. abies*?

Inoculation has a small but significant effect on the local fungal community in the wood (figure 8). One group of fungi which was significantly affected by inoculation status is resinicolous fungi, i.e., fungi which have found an ecological niche in highly resinous substrates (Mitchell 2021). This group had a significantly higher RA in the inoculated wood than in the non-inoculated wood (figure 11). From the photos of the cores, it was also observed that cores from the inoculated wood had significantly larger dark areas than cores from non-inoculated wood, indicating more resinous wood because of inoculation. Resin had been produced near the inoculation point by the tree as a defense response against the pathogen *C. fuckeliana*.

The cores that were photographed and analyzed for dark pixels were not the same cores from which DNA was extracted. Thus, no direct correlation between dark pixels and RA of resinicolous fungi could be done for each sample. The analysis of the dark pixels only indicates that resin content was higher in the inoculated wood than in the non-inoculated. Likewise, the analysis of the RA of resinicolous fungi only establishes that the RA was higher in the inoculated wood than in the non-inoculated.

Another factor to discuss is how resin can affect the extraction and PCR process. If phenolic compounds found in the resin of the wood (Jagadeesh et al. 2022) introduce a bias in the DNA extraction or PCR (Jobes 1995), that could play a part in explaining the small difference in the fungal community found between the treatments. Rather than an actual difference of fungal community, the observed difference would be the bias introduced by higher resin content in inoculated wood than in the non-inoculated wood.

Alpha diversity, measured as SWDI, was observed to be unaffected by inoculation of *C. fuckeliana* (figure 10). It was hypothesized that inoculation would lead to a significant increased abundance of *C. fuckeliana* in the inoculated wood which would dominate the fungal community and reduce alpha-diversity compared to non-inoculated wood. However, much like the SWDI, the RA of *C. fuckeliana* was not significantly different between the samples (figure 11), disproving the hypothesis.

The small effect of inoculation on fungal community (2% of variance explained), suggests that other factors play a role in shaping the fungal community. Distance between trees was tested as an explanatory variable for differences in fungal communities but was only able to explain a negligible part of the variance (figure 9). For wood-dwelling fungi, successful colonization could to a larger degree be affected by the fungus' ability to pass through the bark barrier than by the distance to the source of the inoculum. This fact makes it likely that priority effects (Hiscox et al. 2015) and stochastic events that expose bare wood play a larger role in shaping the fungal community in the wood in this stand, especially considering the small size and homogeneity of the stand.

## 4.5 Implications for forestry and biodiversity

In an unmanaged forest, *C. fuckeliana* is of no concern. It is unlikely to cause disease severe enough to kill large trees that are of importance for biodiversity. When disease occurs, it can rather be seen as an event which creates resinous wood, fostering resinicolous fungal communities. Increased resin content in wood has also been shown to slow decomposition rates (Hassan et al. 2024), making the wood more long lived after the tree has died. This recalcitrant deadwood would allow for a longer succession of wood inhabiting fungi, potentially increasing the biodiversity value of the log compared to more easily decomposed deadwood.

The substrate created by the disease could also be important for several insects. *C. pactolana* (Uimari et al. 2018) and *D. micans* (Vasiliauskas et al. 1996) have shown a positive correlation with infection of *C. fuckeliana*, and although these species are not themselves of particular importance for biodiversity (they are in fact considered forest pests), their correlation with *C. fuckeliana* shows the intimate relationship between fungi and insects. The necrotic tissue created in the canker is one substrate that could be of importance for insect biodiversity, and the recalcitrant wood created in the reaction zone is another.

Currently, *C. fuckeliana* does not pose a major threat to production forestry in Sweden. However, it is possible that the warmer and more humid environment predicted for Sweden due to climate change (SMHI 2026) could be more conducive to disease in *P. abies* caused by *C. fuckeliana*. If future climate is also characterized by an increase in tree stressing events like droughts (Canedo Rosso et al. 2025) this could add to the severity of the problem (Sturrock et al. 2010).

Although, since *C. fuckeliana* and *P. abies* have co-evolved for a long time, it is unlikely that damages will be of the same scale as those seen on *P. radiata* in the southern hemisphere.

Based on the earlier studies and finds presented in this thesis, three potential problems that *C. fuckeliana* could cause in the future have been identified. Firstly, trunks of susceptible trees could be deformed by cankers caused by the fungus, reducing the value of the timber. Secondly, adult stands of *P. abies* could experience dieback of branches and twigs like what have been seen with other *Corinectria* species (Pavlov et al. 2020). Thirdly, young *P. abies* trees in sites with environments conducive to disease could be vulnerable to girdling and top dieback, which would negatively affect regeneration. Continuous monitoring of cankers appearing in *P. abies* stands will be important to follow how disease caused by *C. fuckeliana* changes with climate change.

## 4.6 Conclusion and future research

In conclusion, unstressed trees of *P. abies* can manage and survive cankers caused by *C. fuckeliana* for several years. The observed trees were able to contain the disease by allocating resources and increasing resin production at the site of the canker, preventing it from growing and girdling the trunk. The fungus persists several years after inoculation in the wood. Currently, the largest problems caused by *C. fuckeliana* for Swedish forestry is loss of timber value from deformation of trunks, and extensive top dieback of young *P. abies* stands.

Future research of *C. fuckeliana* can be divided into two categories. Firstly, the risk it poses as a forest pathogen should be further analyzed. This could include new inoculation trials on *P. abies* with different climatic treatments and stressing factors, giving insights into how the disease will change with climate change. It can also include sequencing of fungal communities of insect pests known to attack *P. abies* to better understand the role that different insect species play as vectors for the disease. Testing how the genetic component of *P. abies* trees affects the severity of the disease can also be useful if breeding for less susceptible hosts becomes relevant.

Secondly, research into how *C. fuckeliana* affects the properties of the deadwood that is created when the tree dies will help us understand the role that it plays as an agent of fungal biodiversity in unmanaged forests. This can be done by creating deadwood from logs with cankers caused by *C. fuckeliana*. Annual metabarcoding and surveying of sporocarps on these logs would broaden our knowledge of how this fungus and other canker-causing fungi shape the quality of *P. abies* deadwood.

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# Popular science summary

Forests cover much of Sweden and are important both economically and ecologically. Norway spruce (*Picea abies*) is one of the country's most important forestry species, but it is vulnerable to fungal pathogens that can reduce growth and timber quality. This thesis examines the long-term effects of disease caused by the fungus *Corinectria fuckeliana*, a species that for a long time has been considered a weak pathogen but which is now increasingly linked to stem cankers and deformities in Norway spruce.

The study presented in this thesis builds on a field experiment established in 2011, in which spruce trees in Håbo, Uppsala were inoculated with *C. fuckeliana*. Fourteen years later, the same trees were re-examined to investigate how the fungus had affected tree growth, stem quality, and the fungal communities within the wood.

The results showed that infection caused significant growth reduction near the inoculation sites during the first years after infection and the trees developed elongated fluted cankers. Fourteen years later, swelling of the trunk could be seen around the inoculation point. Despite these effects, no trees died from the infection. Over time, the visible lesions became smaller as the trees continued to grow, suggesting that healthy Norway spruce can often contain and survive disease caused by *C. fuckeliana*.

DNA-based analyses showed that *C. fuckeliana* persisted inside the wood many years after inoculation. It was also detected in wood sampled from parts of the trunk without visible symptoms, indicating that it may spread beyond damaged tissue and persist within the wood of living trees. The disease also influenced the fungal community within the wood. Areas around the cankers contained more resin-rich tissue, produced by the tree as part of its defense response. This environment favored resinicolous fungi, species specialized in growing in resin-rich substrates.

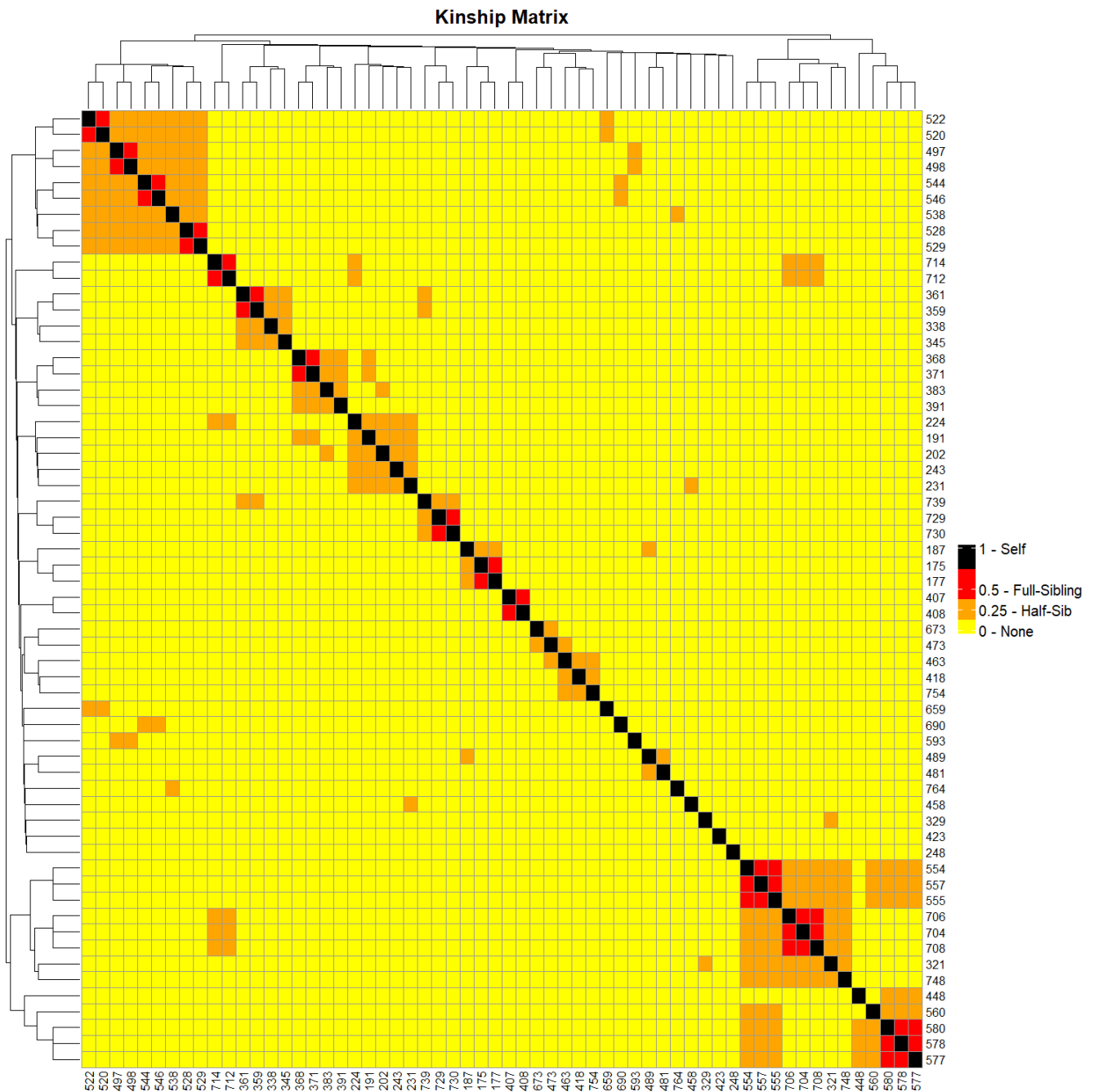
These findings improve understanding of how this fungal pathogen affects Swedish forests. Although *C. fuckeliana* currently causes limited damage for Swedish forestry, warmer temperatures, higher humidity, and increased environmental stress could increase disease severity. The study also highlights the ecological role of fungal pathogens. By creating resin-rich and damaged wood, fungi such as *C. fuckeliana* may contribute to habitats that support other fungi and insects, influencing biodiversity and decomposition processes in forest ecosystems.

# Acknowledgments

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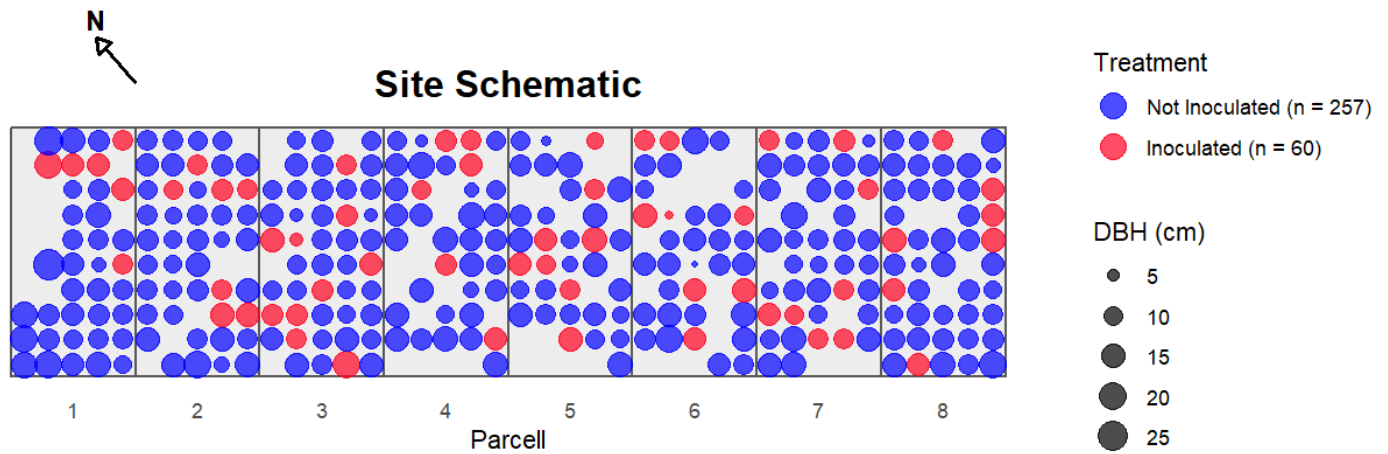
# Appendix 1

Kinship matrix of all inoculated trees. The matrix uses only parental information. Black squares represent clones or self-pairs, red squares share two parents, orange squares share one parent, and yellow squares share no parent.



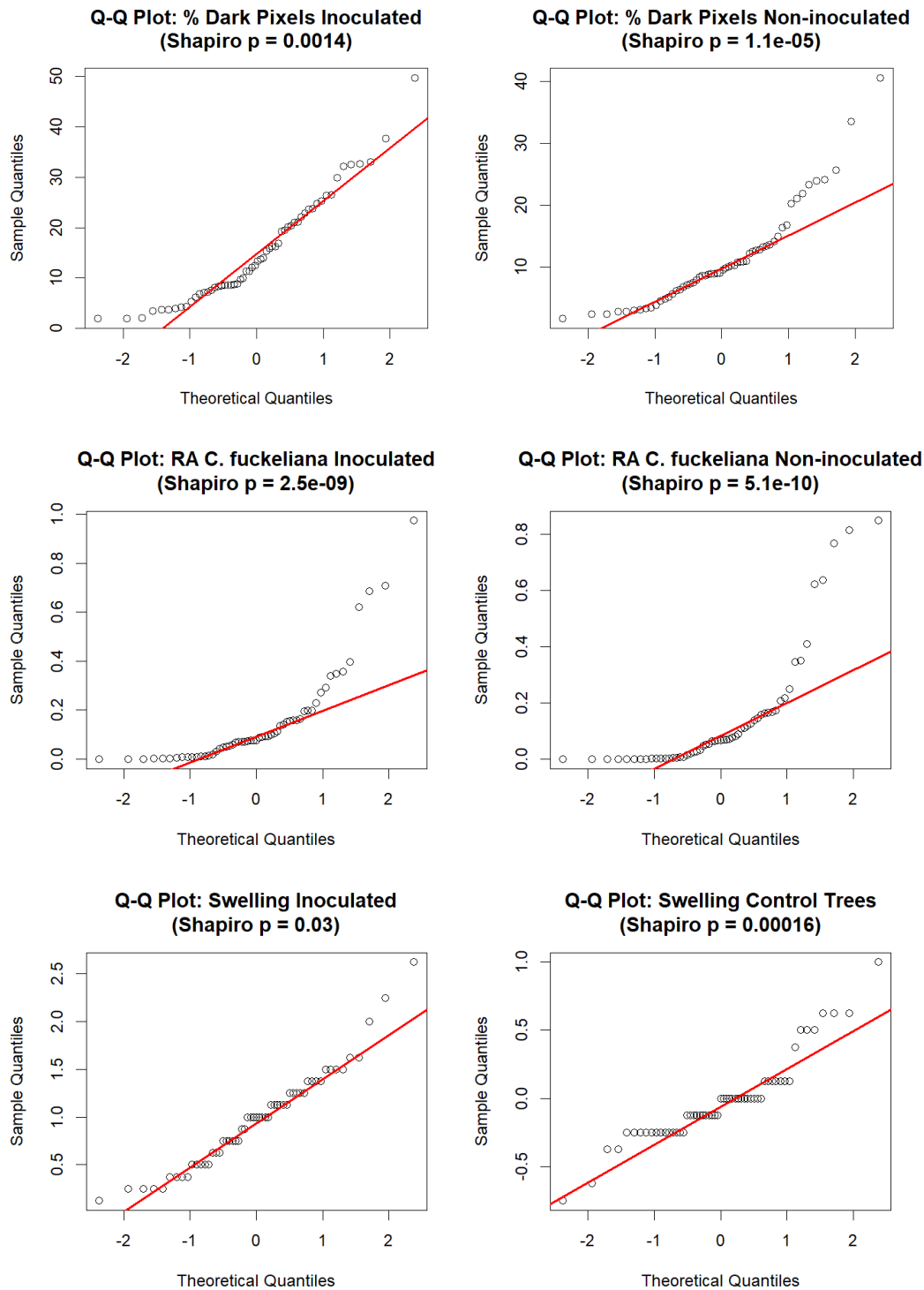
## Appendix 2

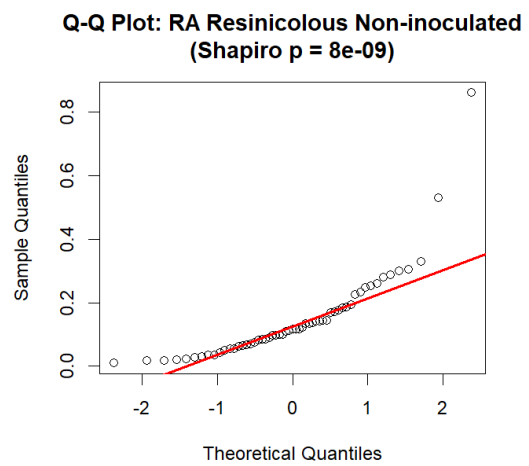
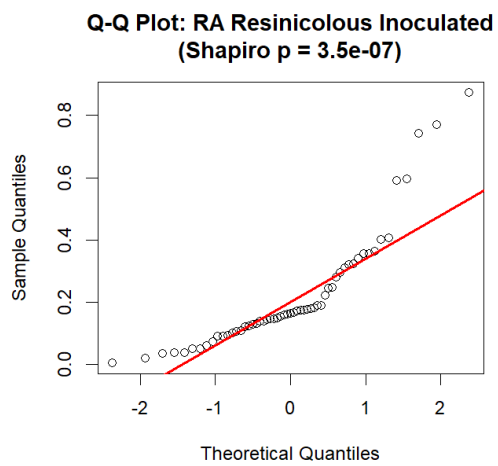
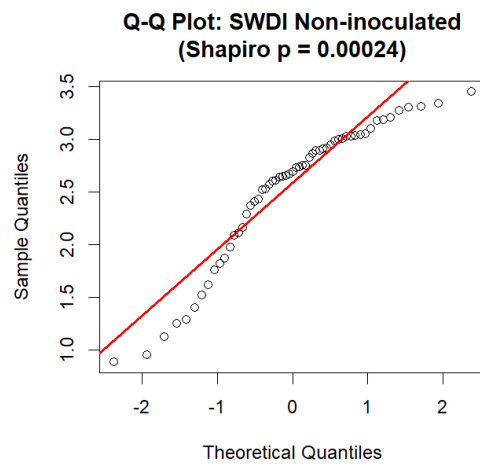
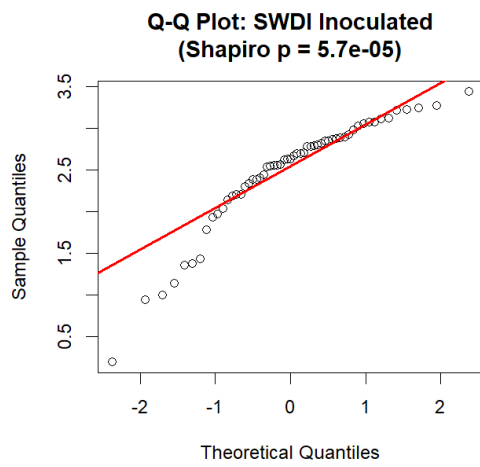
Schematic representation of experiment site showing distribution of diameters and inoculation status within the parcels. Blue dots represent non-inoculated trees and red dot represent inoculated trees. The size of the dot correlates to the diameter at breast height measured in 2025.



## Appendix 3

QQ-plots and Shapiro tests showing non-normal distributions.





## Appendix 4

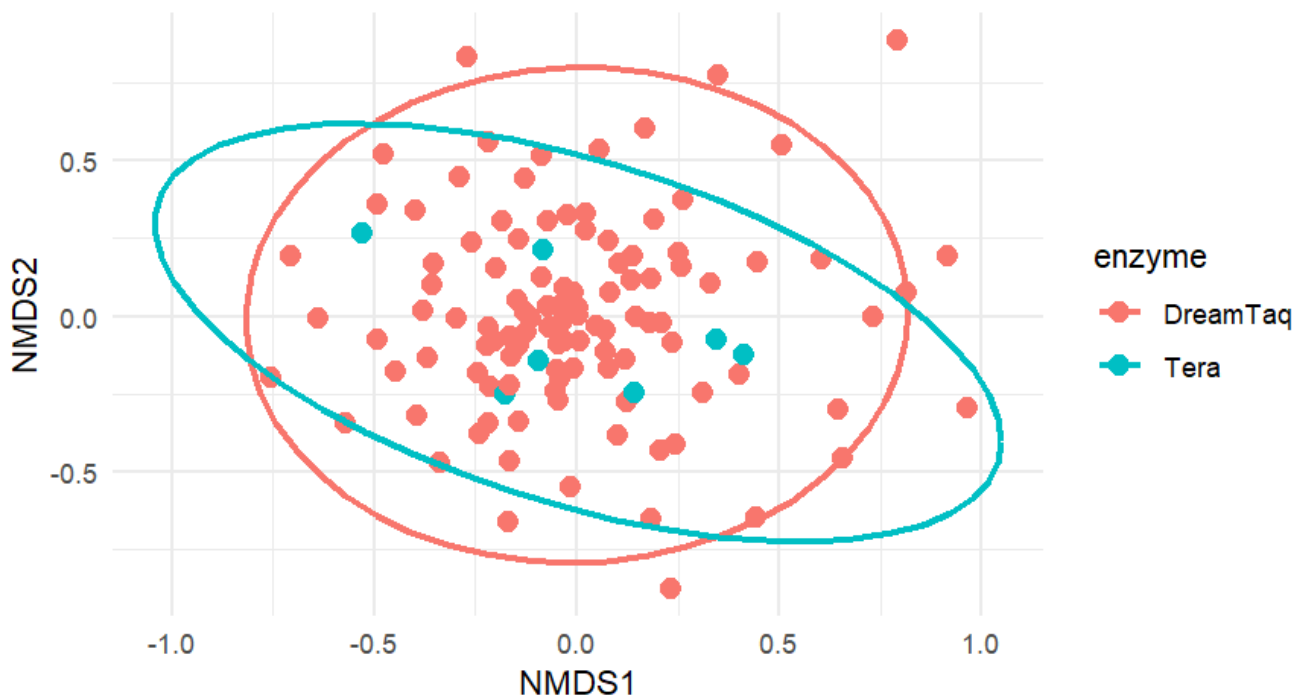
| Name                                            | Description                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Value    |
|-------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| <b>Clustering distance</b>                      | Threshold distance for where clustering should occur. Any sequence which is less than this divergent from some other sequence will be added to the cluster. Divergence is calculated based on pairwise alignments with minimum length according to the option set below.                                                                                                                                                                                       | 0.015    |
| <b>Minimum alignment to consider clustering</b> | Minimum length of pairwise alignment in the clustering process required to consider a sequence pair for clustering. It is recommended to keep this value close to the minimum read length requirement to avoid over-clustering on conserved motif within the sequences. The value can be either an absolute value in basepairs, or a value between 0 and 1 giving the proportion of the longest sequence in a sequence pair that is included in the alignment. | 0.85     |
| <b>Mismatch penalty</b>                         | Missmatch penalty to use in hit scoring during clustering.                                                                                                                                                                                                                                                                                                                                                                                                     | 1        |
| <b>Gap open penalty</b>                         | Gap extension open penalty to use in hit scoring during clustering.                                                                                                                                                                                                                                                                                                                                                                                            | 0        |
| <b>Gap extension penalty</b>                    | Gap extension penalty to use in hit scoring during clustering.                                                                                                                                                                                                                                                                                                                                                                                                 | 1        |
| <b>End gap weight</b>                           | Weighting of end gaps (open and extension) to use in hit scoring during clustering.                                                                                                                                                                                                                                                                                                                                                                            | 0.0      |
| <b>Collapse homopolymers</b>                    | Collapse homopolymers longer than the given length before clustering. A value of 0 will turn this function off                                                                                                                                                                                                                                                                                                                                                 | 3        |
| <b>Downsample sample size</b>                   | If this value is greater than 0, sequences for any sample/tag with more than this many sequences will be randomly downsampled to the given size                                                                                                                                                                                                                                                                                                                | 0        |
| <b>Remove low frequency genotypes</b>           | Remove genotypes from the combined dataset that occurs this many times or less.                                                                                                                                                                                                                                                                                                                                                                                | 1        |
| <b>Tag-by-Cluster Max</b>                       | Maximum number of sequences to include in the tag-by-cluster data matrix of relative cluster abundance.                                                                                                                                                                                                                                                                                                                                                        | 10000000 |
| <b>Blast E-value cutoff</b>                     | E-value filter on BLAST hits in the clustering process. Leave this at the default unless you increase the clustering threshold to several per cent. Higher values will only waste cpu resources and running time. Please check the e_values.txt by plotting e_value against distance file if you suspect that the clustering is truncated due to too stringent e-value filtering. (Applies only to BLAST clustering)                                           | 1,00E-60 |
| <b>Cluster engine</b>                           | What cluster engine to use. Please stick to default                                                                                                                                                                                                                                                                                                                                                                                                            | usearch  |
| <b>Number of repseqs to report</b>              | Report at most this number of representative sequences in summary tables                                                                                                                                                                                                                                                                                                                                                                                       | 3        |

## Appendix 5

NMDS ordination of fungal community grouped by enzyme used in PCR reaction. Stress level of ordination, and  $R^2$  and p-value from PERMANOVA display over plot.

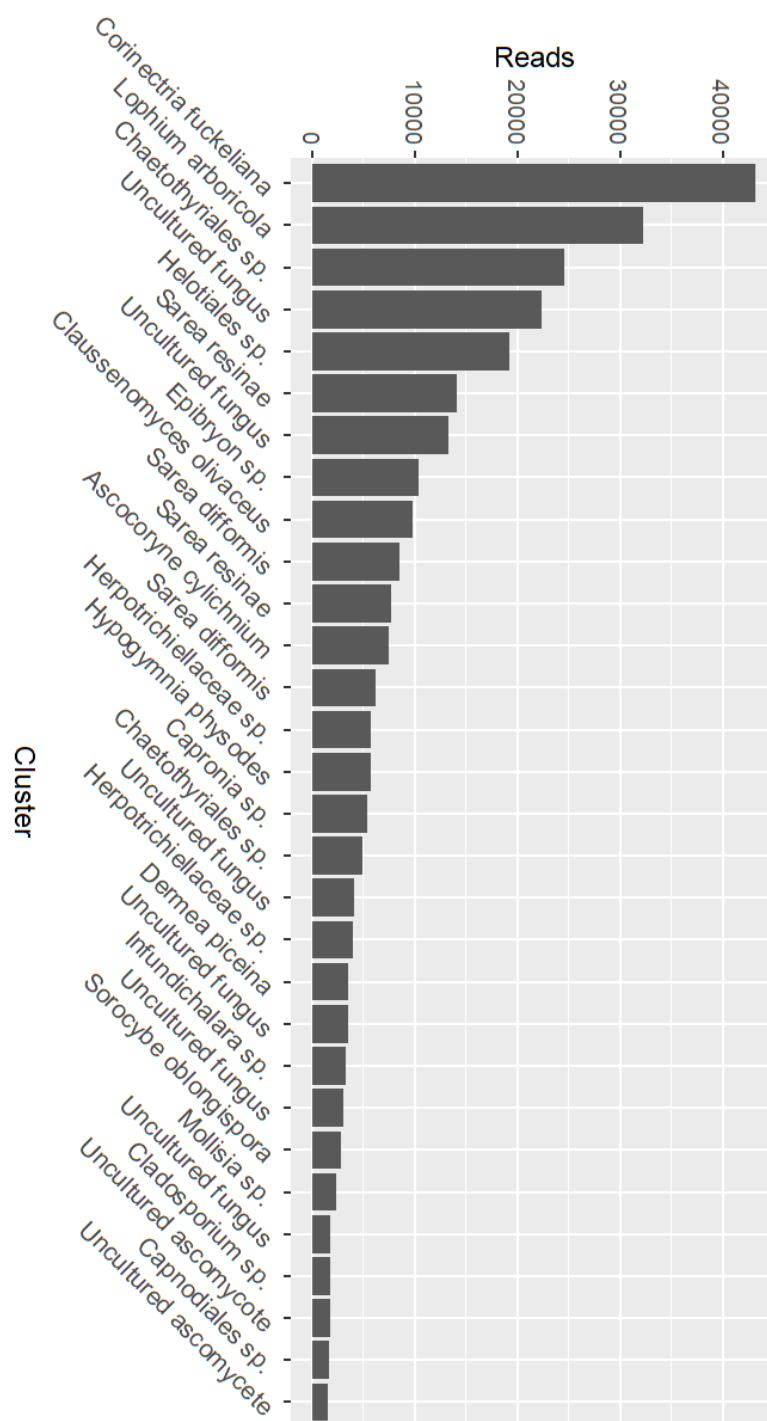
NMDS (stress = 0.282)

PERMANOVA:  $R^2 = 0.008$ ,  $p = 0.565$



## Appendix 6

Species hypotheses and total read count of the top 30 most abundant OTUs.



## Appendix 7

Species hypotheses, accession nr. of reference sequences, and percentage identical match of the top 75 most abundant OTUs

| OTU          | SH                       | Reference sequence | Percent identical |
|--------------|--------------------------|--------------------|-------------------|
| scata6928_2  | Corinectria fuckeliana   | MF593150.1         | 100.00            |
| scata6928_1  | Lophium arboricola       | OM614739.1         | 100.00            |
| scata6928_6  | Chaetothyriales sp.      | LR864113.1         | 100.00            |
| scata6928_3  | Uncultured fungus        | ON750303.1         | 99.61             |
| scata6928_9  | Helotiales sp.           | OR769664.1         | 100.00            |
| scata6928_7  | Sarea difformis          | MH857896.1         | 99.60             |
| scata6928_10 | Sarea resinae            | MN720377.1         | 100.00            |
| scata6928_8  | Uncultured fungus        | OR982287.1         | 98.00             |
| scata6928_5  | Epibryon sp.             | OQ370943.1         | 100.00            |
| scata6928_11 | Claussenomyces olivaceus | KY661432.1         | 100.00            |
| scata6928_13 | Sarea difformis          | MT809287.1         | 100.00            |
| scata6928_16 | Sarea resinae            | MT809245.1         | 100.00            |
| scata6928_14 | Ascocoryne cylichnium    | HM152544.1         | 100.00            |
| scata6928_20 | Hypogymnia physodes      | ON963317.1         | 100.00            |
| scata6928_15 | Herpotrichiellaceae sp.  | MW237935.1         | 100.00            |
| scata6928_21 | Capronia sp.             | MW238163.1         | 99.22             |
| scata6928_19 | Chaetothyriales sp.      | PX690262.1         | 98.43             |
| scata6928_18 | Uncultured fungus        | KT219793.1         | 91.80             |
| scata6928_23 | Uncultured fungus        | OR982295.1         | 98.80             |
| scata6928_22 | Herpotrichiellaceae sp.  | PX690353.1         | 100.00            |
| scata6928_24 | Dermea piceina           | OM614814.1         | 98.33             |
| scata6928_34 | Infundichalara sp.       | PP523959.1         | 97.92             |
| scata6928_27 | Uncultured fungus        | MT266202.1         | 99.57             |
| scata6928_25 | Sorocybe oblongispora    | MN114115.1         | 95.40             |
| scata6928_32 | Arthrographis lignicola  | AB213452.1         | 99.60             |
| scata6928_30 | Mollisia sp.             | OM951666.1         | 100.00            |
| scata6928_35 | Capnodiales sp.          | LR864934.1         | 100.00            |
| scata6928_37 | Uncultured fungus        | KF274291.1         | 100.00            |
| scata6928_36 | Malassezia restricta     | OQ066718.1         | 100.00            |
| scata6928_38 | Cladosporium sp.         | PQ672595.1         | 100.00            |
| scata6928_40 | Uncultured ascomycote    | OX031509.1         | 100.00            |
| scata6928_44 | Uncultured ascomycete    | AM901711.1         | 100.00            |
| scata6928_54 | Exophiala abietophila    | NR_163357.1        | 99.04             |
| scata6928_49 | Uncultured fungus        | MW757649.1         | 100.00            |
| scata6928_52 | Uncultured fungus        | MN902589.1         | 100.00            |
| scata6928_50 | Neocelosporium aereum    | OR680776.1         | 99.61             |
| scata6928_59 | Sarea coeloplata         | MT809285.1         | 100.00            |
| scata6928_53 | Cryptosporiopsis sp.     | OU497898.1         | 97.07             |
| scata6928_55 | Sydowia polyspora        | PP524016.1         | 100.00            |

|               |                                 |             |        |
|---------------|---------------------------------|-------------|--------|
| scata6928_43  | Uncultured fungus               | MF347886.1  | 95.83  |
| scata6928_60  | Uncultured fungus               | JQ312753.1  | 100.00 |
| scata6928_70  | Mollisia albogrisea             | PP266380.1  | 100.00 |
| scata6928_61  | Uncultured fungus               | KP897465.1  | 100.00 |
| scata6928_89  | Scoliciosporum sarothamni       | OQ717605.1  | 100.00 |
| scata6928_66  | Umbilicaria sp.                 | OM614846.1  | 100.00 |
| scata6928_56  | Amphosoma sp.                   | OL467359.1  | 96.85  |
| scata6928_62  | Chaetothyriales sp.             | PX689501.1  | 98.13  |
| scata6928_77  | Leotiomycetes sp.               | PP936022.1  | 100.00 |
| scata6928_72  | Cladophialophora sp.            | MW238107.1  | 99.63  |
| scata6928_65  | Uncultured fungus               | EU689230.1  | 98.02  |
| scata6928_84  | Lachnellula resinaria           | MT913605.1  | 98.33  |
| scata6928_73  | Uncultured fungus               | MN902565.1  | 100.00 |
| scata6928_58  | Uncultured fungus               | KM493439.1  | 97.62  |
| scata6928_75  | Teratosphaeriaceae sp.          | JF449736.1  | 100.00 |
| scata6928_91  | Uncultured fungus               | MN902896.1  | 100.00 |
| scata6928_87  | Parmelia sulcata                | OR589562.1  | 100.00 |
| scata6928_97  | Pucciniomycetes sp.             | MT431342.1  | 99.34  |
| scata6928_96  | Trybliopsis pinastri            | JF793678.1  | 99.59  |
| scata6928_64  | Naganishia sp.                  | PV716659.1  | 100.00 |
| scata6928_98  | Myriangiaceae sp.               | PX690414.1  | 98.79  |
| scata6928_71  | Uncultured fungus               | AM084500.1  | 97.54  |
| scata6928_79  | Cystobasidiomycetes sp.         | MN654832.1  | 100.00 |
| scata6928_80  | Epicoccum nigrum                | OQ411109.1  | 100.00 |
| scata6928_101 | Chaetothyriales sp.             | HQ607871.1  | 96.88  |
| scata6928_90  | Tympanidaceae sp.               | PX690179.1  | 100.00 |
| scata6928_104 | Uncultured fungus               | KU061857.1  | 96.72  |
| scata6928_69  | Gloiothela citrina              | MZ159666.1  | 99.14  |
| scata6928_124 | Uncultured Ascomycota           | KJ827040.1  | 99.15  |
| scata6928_109 | Aotearoamyces sp.               | NR_164216.1 | 93.85  |
| scata6928_141 | Uncultured fungus               | KU063901.1  | 98.35  |
| scata6928_103 | Uncultured fungus               | OM614822.1  | 100.00 |
| scata6928_113 | Aotearoamyces sp.               | NR_164216.1 | 95.92  |
| scata6928_143 | Uncultured fungus               | ON750996.1  | 98.39  |
| scata6928_111 | Claussenomyces kirschsteinianus | KY689628.1  | 100.00 |
| scata6928_127 | Dothideomycetes sp.             | ON122470.1  | 100.00 |

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