

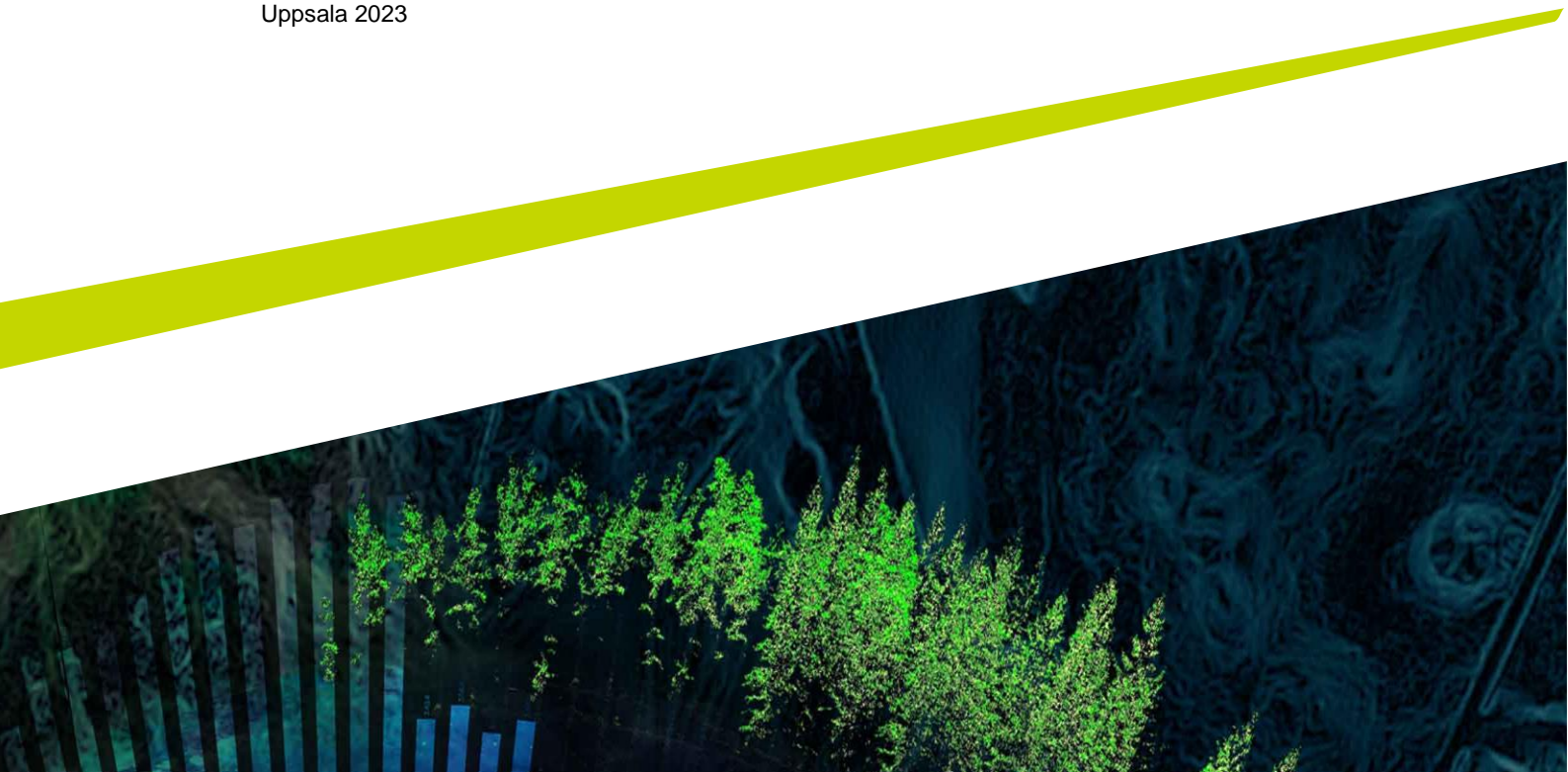


Ectomycorrhizal fungi community shift along gradient from forest to clearcut

Two field seasons after harvest of old growth
Scots pine forests

Linda Johannesson

Degree project/Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Forest Mycology and Plant Pathology
Master thesis in Biology, A2E
Series title, Part number • ISSN XXXX-XXXX (if any)
Uppsala 2023



ECM community shift along gradient from forest to clearcut

Two field seasons after harvest of old growth Scots pine forests

Linda Johannesson

Supervisor: Anders Dahlberg, SLU, Swedish University of Agricultural Sciences

Examiner: Åke Olson, SLU, Swedish University of Agricultural Sciences

Credits: 30

Level: A2E

Course title: Master thesis in Biology

Course code: EX0895

Course coordinating dept: Forest Mycology and Plant Pathology

Place of publication: Uppsala

Year of publication: 2023

Copyright: All featured images are used with permission from the copyright owner.

Keywords: biodiversity, ectomycorrhiza, fungi, retention trees, clearcutting

Swedish University of Agricultural Sciences

Forest Mycology and Plant Pathology

Abstract

It is known that the number of ECM decline and the community composition is altered by clearcutting. This study aims to investigate impacts at a more detailed scale. This study investigates 1) how the ECM community (composition and species richness) change along a gradient from old growth Scots pine (*Pinus sylvestris*) forest to 30m out on 2-year-old clearcuttings. 2) and how the ECM community (composition and species richness) change along a gradient reaching 30 m from retention trees left on the same clearcuts. This is achieved by extracting fungi DNA from 120, respectively 75 soil samples, from 4 sites in Dalarna County, Sweden.

The study shows that the number of ECM species is lower on 1–2-year-old clearcuts of old growth pine forest, compared to intact old growth pine forest, and that the number of ECM species decline along the 30 m long gradient from the forest edge. The proportion of ECM abundance compared to total fungal abundance remains constant along the same gradient. The study shows that the number of ECM species and proportion of ECM abundance compared to total fungal abundance decline along 30 m long gradients from single retention trees. The ECM species composition changes significantly with the distance from the forest edge, the change occur between 3-7 m. The ECM species composition did not change with the distance from single retention trees.

The study shows that clearcutting affects fungal communities associated with old-growth forest and that single retention trees can lifeboat a few more species compared to a clearcut without retention trees but have a small effect in preserving fungal communities associated with old growth forest in clearcuts. These findings implies that preservation of ECM diversity associated with later stages of forest succession require a higher level of forest tree continuity than clear-cut management within forest management of today results in.

Keywords: biodiversity, ectomycorrhiza, fungi, retention trees, clearcutting

Table of contents

1.	Introduction	7
1.1.1	Clearcutting and biodiversity.....	7
1.1.2	How ECM species respond to clearcutting.....	8
1.1.3	Declining ECM-populations in Sweden	10
1.1.4	How far from trees can ECM species survive?.....	11
1.2	Aim of the study	12
2.	Material and Methods	13
2.1	Study sites and fieldwork	13
2.1.1	Study sites	13
2.1.2	Soil sampling	16
2.2	Labwork.....	20
2.2.1	Freeze-drying samples	21
2.2.2	Homogenizing the samples	21
2.2.3	DNA-extractions.....	21
2.2.4	Measuring concentration	21
2.2.5	Sample dilution and PCR.....	22
2.2.6	Gel electrophoresis	22
2.2.7	Purification and measurement.....	23
2.2.8	Sequencing.....	23
2.3	Analysing the sequenced data.....	23
2.3.1	Taxa identifications	23
2.3.2	Sorting out ECM species	24
2.3.3	Statistical analyses	24
3.	Results	26
3.1	Species richness and proportion of ECM-reads in relation to distance	27
3.1.1	Forest edge.....	27
3.1.2	Retention trees	29
3.2	ECM-community composition in relation to distance	30
3.2.1	Forest edge.....	30
3.2.2	Retention trees	32
3.3	Overall observed ECM-community structure	33
3.4	Red-listed species.....	37

4. Discussion	38
4.1 ECM species richness and distance	38
4.2 ECM-community composition and distance	39
4.3 Overall observed ECM-community structure	40
4.4 Red-listed species	41
4.5 Conclusion	42
References	43
Invisible underground diversity on clearcuttings?	52
Acknowledgements	54
Appendix 1	55

1. Introduction

1.1.1 Clearcutting and biodiversity

During the last century, forests have been converted from natural to managed stands to a notable extent in northern Europe (Östlund et al. 1997, Ahlström et al. 2022). The practice of clearcutting as a forestry method have gradually become the dominant practice (Lundmark et al. 2013, Kuuluvainen et al. 2012). In some countries, almost all productive forest land has been subject to the forestry practice clearcutting. For example, in Sweden, at least 89% of the managed forest land is managed by the clearcutting method (Mason et al. 2022). This stand replacing way of forest use is different from the natural occurring disturbances. In northern Europe, natural disturbances have generally taken place on a smaller spatial scale, resulting in a landscape characterized by a more continuous forest cover, and forests with a more complex forest structure featuring large, old trees and versatile dead wood. (Kuuluvainen 2009). This decline in complexity have led to a loss off biodiversity, many populations of species associated with forest habitat have declined and will likely continue to decline if old growth forests continue to be clearcut (SLU Artdatabanken 2020, Hyvärinen et al. 2019). Populations of some species will probably continue to decline according to the theory extinction debt (Figueiredo et al. 2019). This would at least be true for species that has dispersal limitations, and have difficulty to disperse in a fragmented landscape, where suitable forest habitats occur as small “islands” in a landscape dominated by young forests, agriculture land, or other human created environments. Of all red listed species, including near threatened species, in Sweden, 1400 has felling as a main reason for their status as red listed (Eide 2020). It is, however, important to keep in mind, that there is a vast knowledge-gap in terms of species knowledge. This is especially true for organism groups with a large number of inconspicuous species, such as soil-dwelling fungi (Hawksworth 2017). Over the past 20-30 years there has been a growing emphasis on mitigating the adverse impacts of forestry on biodiversity (Skogsstyrelsen 2023). In order to know how different mitigating methods would benefit a variation of biodiversity the most, it is crucial to know how different organism groups reacts to these types of land-use. Here, ECM (ectomycorrhizal) fungi are an interesting group of organisms to study, because of their symbiotic relationship with their host trees.

Although there are studies that have reported that the number of ECM and the community composition changes when forests are clearcut (Djupström 2022, Sterkenburg et al. 2019, Varenius et al., 2016), there are still more knowledge left to gain on this topic. For example, if the same results can be observed at different sites geographically, and in forests with different tree-species composition, and if the same results can be obtained for clearcuts of different sizes.

Up until quite recently, only a few studies have been done on how well retention trees manage to sustain mycorrhiza populations (Gustafsson et al. 2016). The studies that have been done on retention trees in Scots pine (*Pinus sylvestris*) forest have focused more on how a different retention tree level affects the mycorrhiza community (Sterkenburg et al. 2019, Djupström et al. 2022). The only study on single retention trees of Scots pine, looks on the difference in community close to the tree, compared to the community on the clearcut far away from retention trees (Varenius et al. 2016). There are no studies on how the community changes along a gradient from single retention trees of Scots pine.

1.1.2 How ECM species respond to clearcutting

Almost 90% of the worlds vascular plants consistently form symbiotic associations with one or more mycorrhizal types, 2% of the vascular plants form ECM associations (Brundrett & Tedersoo 2018). Many of the species form large sporocarps, but there are also many of them that form inconspicuous fruiting bodies (Köljalg et al. 2000). There are approximately 7750 known ECM species in the world, (Rinaldi et al. 2008), distributed on 251 genera (Tedersoo & Smith 2013), of which about 1500 species can be found in Sweden (SLU Artdatabanken 2023a). In Sweden there are currently 341 red-listed mycorrhiza-species, 290 of these are tied to forest habitat. In IUCN's global Red List published in 2022, 50% of the evaluated fungal species, both ECM and non-ECM species, ended up as red-listed (Mueller et al. 2022).

If the host tree is removed, the mycelia of ECM species will die off rather quickly if there are no other living roots in its vicinity which it also colonizes. This as an effect of the cease of supply of sugars from the host (Högberg et al. 2021). On the other hand, an ECM individual may potentially reach old ages as long as suitable host trees are continuous present. The age of a single ECM individual is hard to study, but models based on the growth speed suggest that a mycelium from a single individual can reach similar age as trees, possibly even older (Dahlberg & Mueller 2011).

How well ECM species may survive disturbances of their environment, such as clearcutting, correspond to their ability to survive during the change in the environment and how well they disperse and recolonize once the forest grows of age again. ECM species can survive a clearcutting event, if there are seedlings or trees which it already colonizes left on the clearcut. Otherwise, they are dependent of recolonization by spores from the surrounding. To what extent this is successful will be determined by properties of a species itself, how good a certain species is at producing sporocarps, how much spores these produce, how well these disperse to suitable habitat in the surrounding, how far the spores spread, how long the spores can survive without a host plant, how long the spores can withstand abiotic factors such as drought. And it will also be determined by the properties of the landscape. How fragmented is the landscape? Re-colonisation is less likely to occur if the spores need to cross large distances to reach suitable habitat. And if the isolated forest “islands” are few, it is even less likely that spores of different species will reach the islands since the spore pressure is simply too low. If there are few fruiting bodies of a certain species in the landscape, it is a little chance that this species spores will spread to islands far away.

Different species produce different amount of sporocarps, and the biomass of the mycelia does not correspond to the amount of sporocarps that are produced (De la Varga et al. 2016). A mycelium does not necessarily produce sporocarps yearly. Several years can pass in waiting for suitable weather conditions before the mycelium it produces any sporocarps (Straatsma & Krisai-Greilhuber 2003). Spore dispersal can be mediated either by wind or by animals. Species that produce sporocarps underground are generally dispersing their spores via animals, but it is known that species that produce sporocarps above ground can take help from animals as well (Vašutová et al. 2019). Little is however known on this topic. There has been a general belief that spore dispersal via wind is not distance limited, small spores should be able to disperse essentially any distances by wind due to their small sizes. There are however studies that have obtained the opposite results. In a Californian study, they found that ectomycorrhizal fungi have the potential to disperse and colonize over multiple kilometres. However, at distances over a kilometre or greater, a study showed a significant proportion of bait seedlings remained uncolonized (Peay et al. 2012). Even if some spores can cross vast distances, most of the spores produced, 95%, end up just beneath or within a few meters from the sporocarp (Li 2005, Galante et al. 2011). Another Californian study showed that single trees in an alpine environment with a mean age of 65 years the ECM species richness declined with distance to the forest edge. This study also found that the ECM species richness increases with the size of the trees (Glassman 2017). These studies points in the direction that the theory of island biogeography applies on ectomycorrhizal fungi. Additionally, there is a trade-off between

competition and colonization for ECM species, meaning that the abundance of each species will not peak at the nearest suitable location, but at the nearest location within its dispersal range at which it can escape superior competitors (Smith et al. 2018). Since there are different niches to fill regarding different dispersal mediators, length of dispersal, and competition ability, different ECM species have different dispersal ability. Essentially different ECM species have different ability to disperse. And the order in which ECM species happen to disperse to and colonize a new site, influence the fungal community assemblage later on (Peay et al. 2012).

1.1.3 Declining ECM-populations in Sweden

Scots pine is alongside Norway spruce and birch the dominating tree species in Sweden. Based on an evaluation of 1100 mycorrhiza species, around 40% is able to form mycorrhiza with Scots pine, and about 10 % form mycorrhiza with Scots pine only (Hallingbäck & Aronsson 1998, Dahlberg et al. 2001). There are currently 62 red listed mycorrhiza species that are associated with Scots pine (Artfakta 2023b). The Scots pine dominated forests in the northern hemisphere have historically been a subject to wildfires regularly. In a Swedish study on a Scots pine dominated forest area of 19 X 32 km, found that approximately 0.8% of the forest was burned annually prior to 1650, and that this rate increased to 2.8% in the mid-1800s. This increase is linked to the expansion of human settlements, prior to that, lightning was the main cause for forest fire (Niklasson & Granström 2000). The fires in Swedish ecosystems have generally been of low intensity, where most of the trees survive, and a significant proportion of the organic soil layer, where the majority of mycorrhizas are located, is left intact (Barker et al. 2013). Contrary, when high intensity fires that kills a large proportion of the trees and occur, it will likely kill all the mycorrhiza as well (Dahlberg et al. 2001). The soil profile of Scots pine dominated forests are of podzol type. The uppermost litter horizon is typically dominated by saprotrophs and fungi with unknown ecology. In boreal forests ECM species dominates both the organic and mineral soil horizons (Carteron et al. 2021, Santalahti et al. 2016). Typically, most ECM species can be found in the organic horizon, where the root density also is the highest. However, for Scots pine, about 2/3 of the root volume is found in the mineral soil, and a study conducted in Finland found that some ECM species that occurred in the mineral soil horizon could not be found in the organic soil layer (Santalahti et al. 2016). Horizontally the ECM mycelial distribution is limited by the length of the host tree roots. For Scots pine, the roots can typically reach about 10 meters (Kalliokoski et al. 2008).

1.1.4 How far from trees can ECM species survive?

A clearcutting will result in an almost complete loss of ECM as a result of a vanished supply of sugars from the host trees (Högberg et al. 2021). However, after plantation, ECM will recolonize the new tree roots, but in comparison with older stands which are dominated of late successional taxa as *Cortinarius* and *Russula* the younger stand will be dominated by pioneer taxa such as *Atheliaceae* (Kyaschenko et al. 2017). The changes in ECM community after clearcut are still palpable for at least 50 years, but as long as there are forest habitats close enough, from which the fungi can recolonise, the stand may become more similar to old natural stands with increasing stand age (Kyaschenko et al. 2017, Varenius et al. 2017). There are some studies on the effect of retention trees on mycorrhizal community. One study which investigated the mycorrhiza colonising seedlings around single Douglas firs (*Pseudotsuga menziesii*), found that there was a higher diversity and evenness of the mycorrhizal community for seedlings within < 6 m of the mature tree, compared to seedlings at a distance range of 16-30 meters from the mature trees (Cline et al. 2005). Another study on Douglas firs noted a shift in ECM community structure at distances >5 m from retention trees within 1-2 years after harvest, and a 50% declination of the number of ECM species at 8-25 m from the retention trees (Luoma et al. 2006). Results obtained in a study which investigated the effect of different percentages of thinning of Scots pine forests in Sweden. In a study, made five years after a thinning event, the mycorrhizal community changed with the distance to the nearest retention tree, where the largest mean distance between trees was 14 meters (Djupström et al. 2022). In another study, sampling 1 m in diameter around retention trees of Scots pine in 10–60-year-old clearcuts in Sweden, obtained the result that the ECM community was partly maintained compared to that in the surrounding old growth forest (Varenius et al. 2017). However, the retention trees made no difference for the ECM community in the surrounding clearcut. These studies point to the direction that retention trees may sustain a mycorrhizal community similar to a community of an old growth forest, within a few meters around single retention trees.

To further understand how the ECM community is affected by clearcutting, and supposedly mitigating measurements such as decreasing the size of the clearcuttings or the leaving of retention trees, more studies should be conducted. It is for example of interest to see if the same results can be obtained at other study sites, with different stand characteristics, and to know in more detail if these results can be seen within a shorter time after felling.

1.2 Aim of the study

The aim of the study is to investigate 1) the characteristics of ectomycorrhizal community (composition and richness) along transects from old growth Scots pine forest to 30m in 2-year-old clearcuttings and similarly 2) the along a gradient reaching 30 m from retention trees left on 2-year-old clearcuttings. In addition, if any red-listed species are found, their spatial occurrences will be investigated.

The hypothesis is that the ectomycorrhizal community will be affected with the distance to the forest edge, and with the distance from the retention trees. Both the number of ECM species, and the proportion of ECM species of all fungal species will decline with increasing distance from forest edges and retention trees. I hypothesise that I will see a drop in the number of species and that the species composition will change along a gradient from the forest edge and the retention trees.

2. Material and Methods

2.1 Study sites and fieldwork

2.1.1 Study sites

The clearcutting to be used as study sites were selected by Stora Enso. The requirements formulated by the study to be met were:

- 5 clearcuttings within Dalarna County,
- felling occurred during the winter 2020-2021,
- Scots pine on moraine-soil with a site productivity about T20,
- location next to an old growth forest at least 120 years, preferably with as similar properties, regarding for example species composition, soil type, productivity, and age, compared to the felled forest as possible.



Figure 1 The location of the four study sites Hyckjeberg, Gopbrunn, Söderryssan and Älgsjön in Dalarna County, Sweden..©Lantmäteriet

In total five clearcuttings were selected with sizes ranging between 1,5-2,7 ha. One of the suggested clearcuttings had a far too narrow shape, it would not have been possible to lay out transects without them being too close (closer than 15 m) to the opposite forest edge. Thus, this clearcutting was excluded, and only those with a proper size and shape were included (Fig 1). The clearcuttings were named as follows: Hyckjeberg (2,7 ha), Gopbrunn (1,7 ha), Söderryssan (1,5 ha), and Älgsjön (1,9 ha) (Table 1, Fig 2-5). The clearcutting Hyckjeberg is located in a south faced and rather steep slope. This site is also quite stony and has a productivity of T20. At the timepoint for the sampling, the site had already been scarified and planted with pine seedlings that measured about 1 dm high. The planting had to a large extent been unsuccessful, a large proportion of the seedlings had perished. A larger area around had been felled a few years earlier, leaving a small border to the east and the west of the study site of old growth forest where sampling could take place. The patch of old growth forest west of the clearcutting has an estimated stand age

of 157 years. The older Scots pine-trees were in field estimated to be around 140 years. The old growth forest patch to the east of the clearcutting has a similar age. Both the clearcutting and the old growth forest patch to the west of the clearcutting were fertilized 1998. The old growth forest south of the site consisted of rather wet, spruce- (*Picea abies*) dominated forest, with a higher productivity, and was hence not considered suitable for sampling. Gopbrunn is located in a larger, slightly hilly, rather stony, landscape characterized by old-growth Scots pine forest that historically have been affected by wildfire. Pine trees with an estimated age of 180 years and clearly visible marks from the last fire-episodes and both burnt logs and high stumps is a fairly common sight in the forest surrounding the clearcutting. The stand age of the old growth forest is by Stora Enso estimated to be 114 years, but this is likely an underestimation. The clearcutting has a productivity of T16, and the surrounding old growth forest where transects were laid out has a productivity of T14. Both the clearcutting and the old growth forest was fertilized 1988. At the timepoint for sampling, the clearcutting was planted with Scots pine, but had not been scarified. The site Söderryssan differs from the other three sites by being far less stony. Portions of the ground in this clearcutting is also rather wet. The productivity of the site is T20 or T22 depending on the data source. An old railway embankment runs straight through the clearcut. The surrounding forest where transect number 4 was laid out has a stand age of 100 years according to Stora Enso, and is dominated by Scots pine, with the oldest trees reaching an estimated age of 140 years, with elements of spruce, and scattered birches (*Betula sp.*) and few scattered goat willows (*Salix caprea*). The forest where the rest of the transects were laid out has similar properties. Älgsjön is a narrow clearcutting, about 60 m wide, with a small gravel-road on one side, and a mire on the other. It has a productivity of T18. There is no data on any fertilization. The surrounding old-growth forest in the ends is a mixed Scots pine and spruce forest. The age of the oldest pines is by sight estimated to be around 130 years.

Tabell 1 Stand properties for the studied sites.

Site	Productivity	fertilized	scarified	Signs of wildfire
Hyckjeberg	T20	1998	Yes	No
Gopbrunn	T14-T16	1998	No	Yes
Söderryssan	T20-T22		No	No
Älgsjön	T18		No	No



Figure 2 Foto taken from inside the old growth Scots pine forest, with a view over the clearcut at the site Hyckjeberg.

2.1.2 Soil sampling

Sampling took place September 19-21, 2022. On each clearcutting, five transects were laid out from the forest edge (Figure 2-5). The transects were laid out as far away from each other as possible along the border to the old growth forest. We selected the location of the transects to be representative for the clearcuts and avoided wet areas. And we avoided putting the transects closer to any retention trees than 15m. The border to the old growth forest was in general quite short, which led to the transects end up quite close to each other. The aim was to put one end of the transect 30m into the old growth forest, and then continue with sampling points 1m, 3m, 7m, 15m, and 30m along the transect out on the clearcutting. At each distance, three samples were collected, one on the transect, and two 1m perpendicular to each side of the transect. These three samples were pooled together by collecting the soil together in one bag. In total 90 (30 pooled) samples were collected in each site, resulting in a total of 120 samples. In the same manner as described above, samples were taken along additionally five transects per site from single retention trees and 30 m in a as straight as possible line out from the trees. This gave additionally 100 samples. Samples were taken by using a handheld soil sampler, which consists of a stainless-steel pipe about 5 cm in diameter with a handle in one end, and with a sharpened edge in the other, suitable for cutting of small roots. This end was sharpened using a special tool when needed during the sampling. The soil-sampling tool was pushed down in the soil until the mineral soil was reached (could easily be heard), or when a rock was hit. The sample was then pushed out on a cutting board with the tool designed for this. A knife was used to

cut off all the green parts like mosses at the top, and to cut away any following mineral soil. Pine needles, pinecones, and small sticks were removed by hand. The sample were then transferred to a clean Ziplock plastic bag. The plastic bag was immediately put into a cool bag. The cool bag was stored in the car during the nights. The outdoor temperature never reached below 0°C. During the night September 21-22, the cool bags were stored in a normal fridge. The samples were finally put in a freezer in -80 °C September 22.

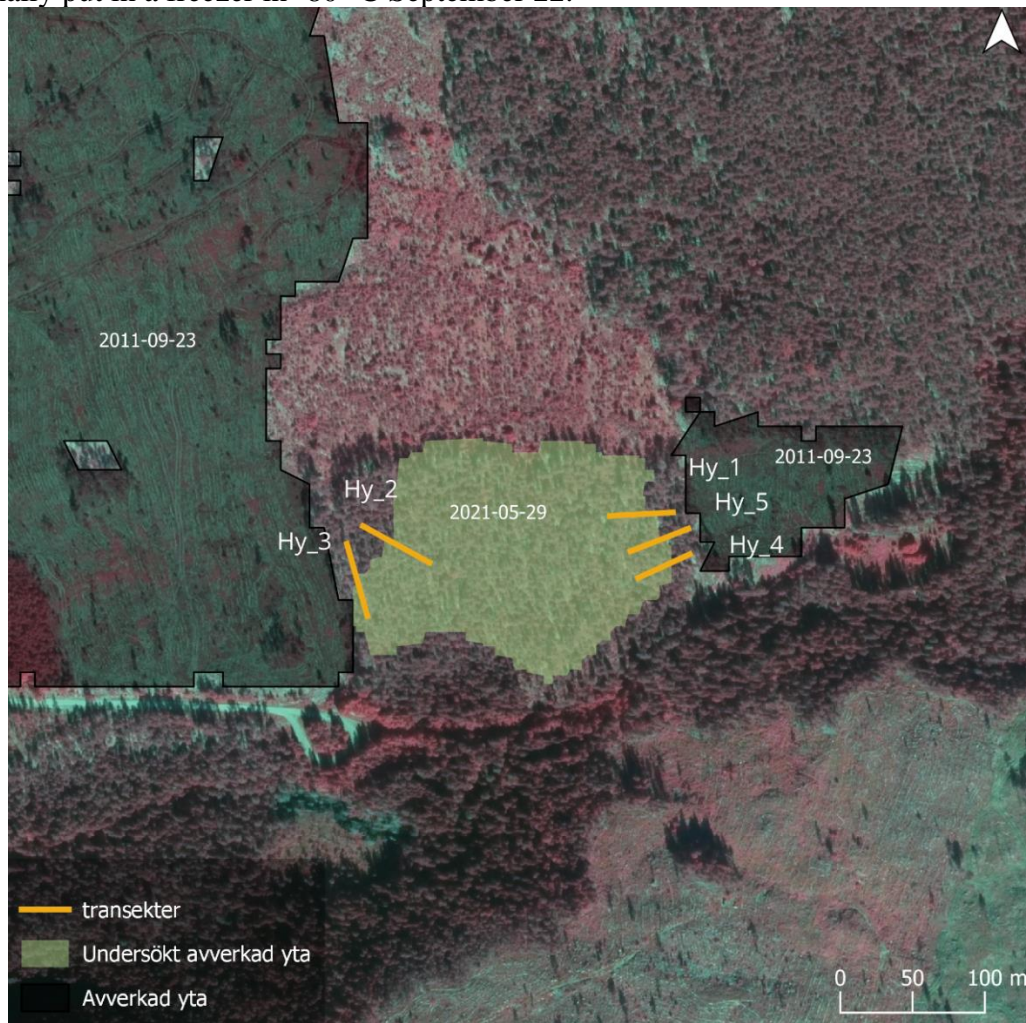


Figure 3 Aerial photograph of Hyckjeberg with the location of the five soil sampling transects marked as orange lines from older forests into the clearcut (light green) felled in 2021. The position

of the sampled seed trees is not shown. Lantmäteriet Ortofoto IRF 0.25/0.50 m latest (tif) is used as background. ©Lantmäteriet.

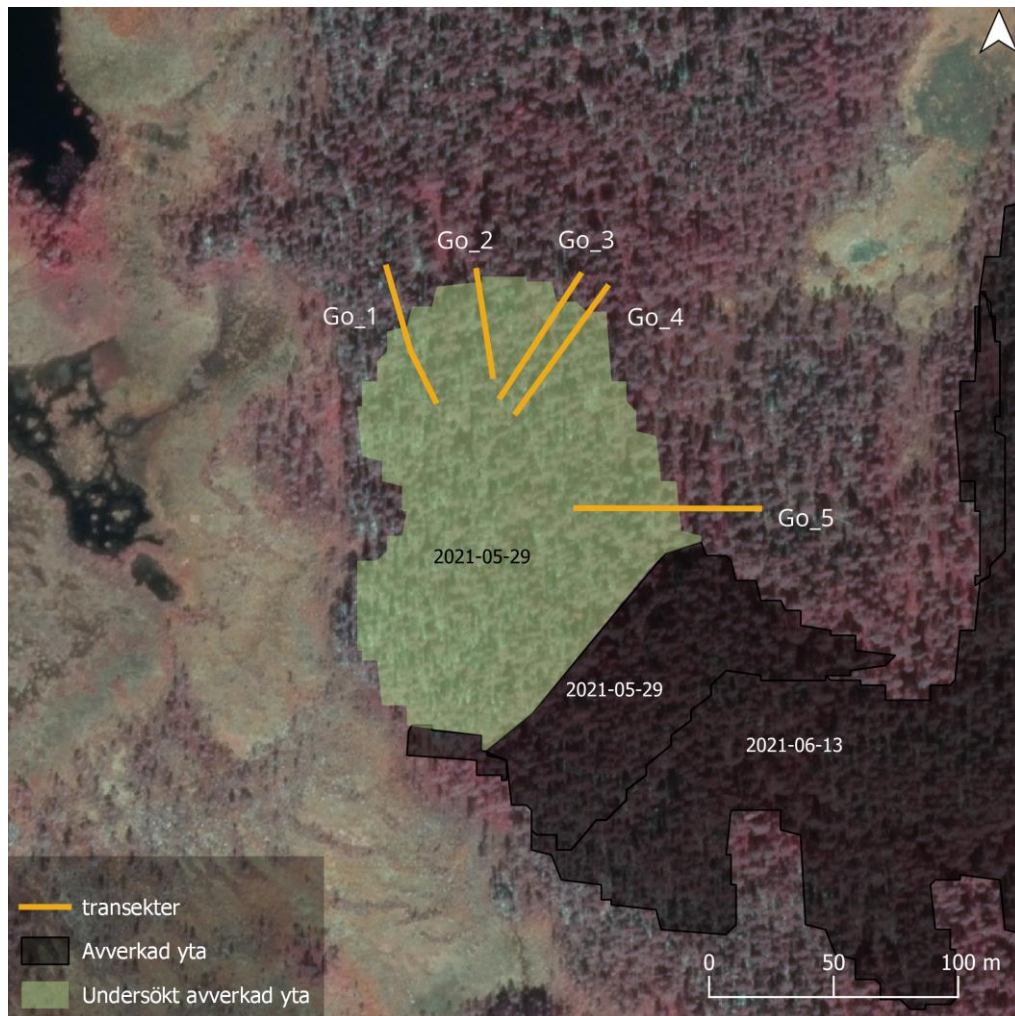


Figure 4 Aerial photograph of Gopbrunn with the location of the five soil sampling transects marked as orange lines from older forests into the clearcut (light green) felled in 2021. The position

of the sampled seed trees is not shown. Lantmäteriet Ortofoto IRF 0.25/0.50 m latest (tif) is used as background. ©Lantmäteriet.

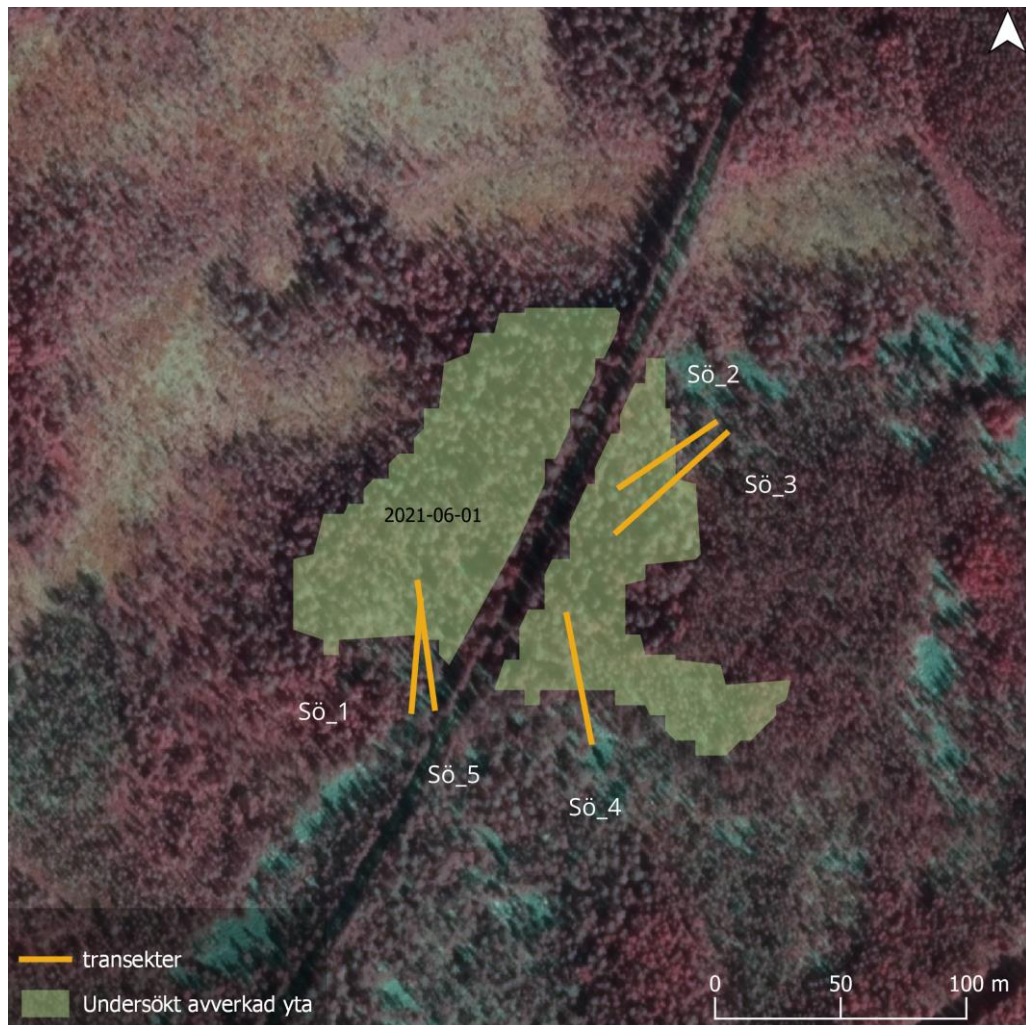


Figure 5 Aerial photograph of Söderryssan with the location of the five soil sampling transects marked as orange lines from older forests into the clearcut (light green) felled in 2021. The position of the sampled seed trees is not shown. Lantmäteriet Ortofoto IRF 0.25/0.50 m latest (tif) is used as background. ©Lantmäteriet.

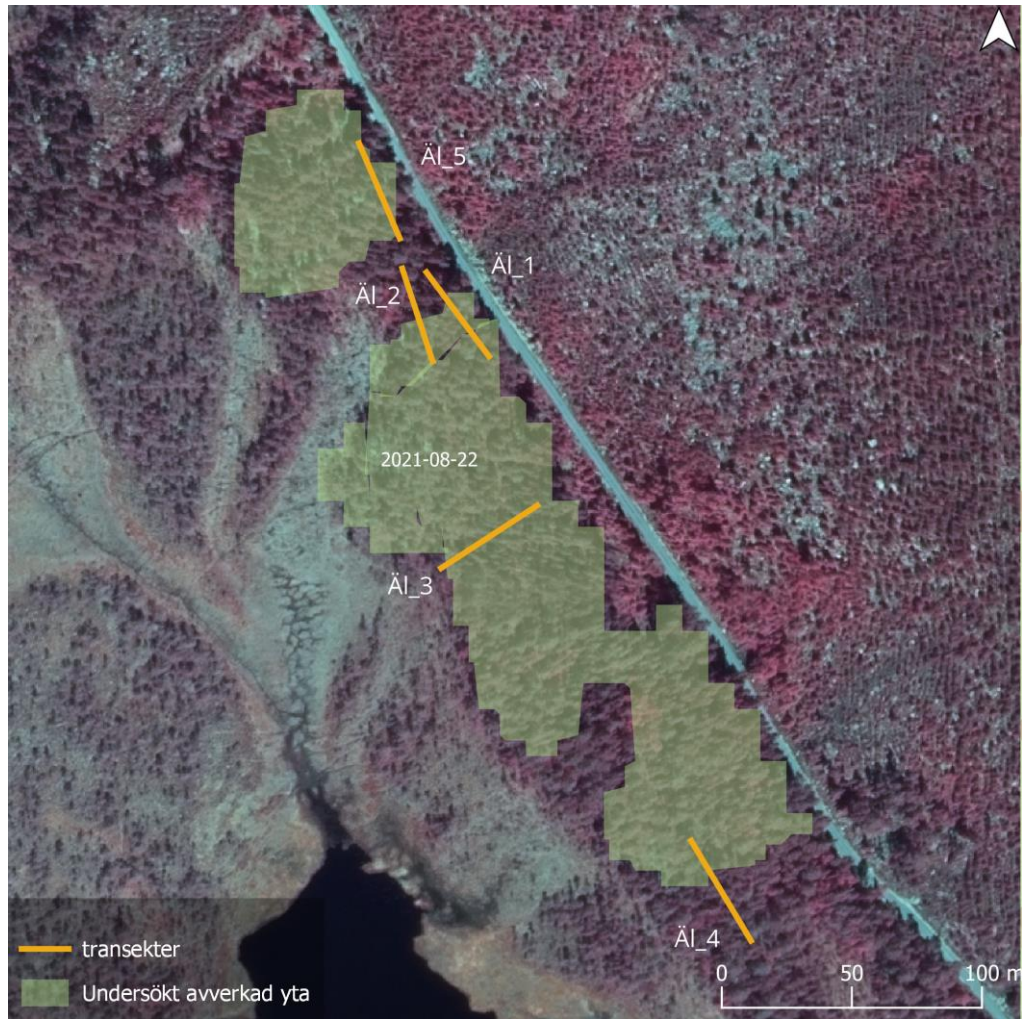


Figure 6 Aerial photograph of Älgsjön with the location of the five soil sampling transects marked as orange lines from older forests into the clearcut (light green) felled in 2021. The position of the sampled seed trees is not shown. Lantmäteriet Ortofoto IRF 0.25/0.50 m latest (tif) is used as background. ©Lantmäteriet.

2.2 Labwork

The laboratory work from freeze drying to gel electrophoresis was done by/ or under supervision of an experienced lab assistant. The final steps of purification and concentration measurements was solely done by the lab assistant.

2.2.1 Freeze-drying samples

The Ziplock-bags with the soil samples were opened and put in a freeze-drier a few at a time until they were fully dry. All samples were then stored in room temperature until further processing.

2.2.2 Homogenizing the samples

The freeze-dried samples were homogenized by removing any pine-needles, mosses, small stones, pinecones, or small sticks that were still left in the sample. Then a mortar was used to manually grind the sample to a fine powder. The mortar was washed by hand and disinfected with 70% ethanol between every sample. For every homogenized sample 1 ml was transferred to an Eppendorf tube. The remaining sample was transferred to a falcon tube, which was saved as a backup. The following samples were still a bit damp about 1-2 weeks after they had been freeze-dried: 174, 177, 178, 182, 183, 191, and 197. These samples were immediately put in the freeze-drier for one more day, until they were fully dried.

2.2.3 DNA-extractions

DNA-extractions were done using the soil-DNA-extraction-kit Macherey Nagel™ NucleoSpin™ (Macherey Nagel™ 2017). Before the extraction process was initiated, all tubes necessary for the different steps of the process was marked. The extraction was done by following the manual (Macherey Nagel™ 2017), except for a few adjustments, that according to experience work better, or gives the same result as by following the manual. In step one, 850 µl SL-buffer was added to 1 ml sample. In step three a FastPrep-24™-machine with the program 2x5000x30 was used. In step four the samples were centrifuged at 13g for 2 min. In step seven, 800 µl sample was transferred to the column. In step ten 80 µl SE-buffer was added to the sample. The final DNA-product was stored in a freezer at -20 °C until further processing.

2.2.4 Measuring concentration

The DNA-concentrations of the samples were measured using a NanoDrop™ spectrophotometer, and by following the manual for the machine and the following computer program. Before any measurements took place, the samples were taken out from the freezer to thaw and were again put back in the freezer again when the measurements were finished.

2.2.5 Sample dilution and PCR

The tubes needed for dilution and for preparing of the mastermix was marked before the PCR-process was initiated. All equipment to be used was disinfected by leaving them under a hood with UV-light on for about 20 min. all the work from here was done under the hood. The samples were diluted to 1/10 by adding 10 μ l DNA-sample to 90 μ l distilled water, the diluted sample was thoroughly vortexed and centrifuged.

The mastermix was prepared using the following protocol (volumes calculated for one sample): 14,9 μ l water, 2,5 μ l reaction buffer, 2,5 μ l dNTP with the concentration 25 mM, and 0,1 μ l Taq-polymerase with the concentration 5U/ μ L. When the mastermix was prepared, 20 μ l was added to each tube in the PCR-plate. Then 2,5 μ l primer, and 2,5 μ l of the diluted DNA was added in the named order. Everything was mixed by pipetting up and down a few times.

In the PCR-machine, a program was used which in step one held 95 °C for 5 min, step two was from the beginning programmed on 35 cycles, but after evaluation of the amount of DNA-product in the gel-electrophoresis, 31 cycles were used instead. The step two of the program started with 95 °C for 30s, then 56 °C for 30 s, and then 72 °C for 30 s. Step three was programmed to hold 72 °C for 7 min, and finally the temperature was lowered to 15 °C. The final PCR-product was stored in a freezer until further use.

2.2.6 Gel electrophoresis

An agarose-gel was prepared by mixing 2,2 g agar with 220 ml SB-buffer a glass-container, and heating it together in a microwave at 700 W for 7 min. Then 5 μ l of the colouring-liquid Nancy-520 from Sigma-aldrich was added and mixed with the hot liquid. After a few minutes of cooling down, the liquid was poured into a gel-tray, that had been prepared by taping the ends to prevent leakage and placing combs. The gel was left to solidify in the tray and meanwhile, the samples were taken out from the freezer to thaw.

When the gel was solidified, and the samples thawed, 5 μ l GR-ladder was added to the wells at the ends of each well-row. 5 μ l of each sample was then added to the rest of the wells. The electrophoresis was performed by keeping 300 V for 20 min. The gel was scanned using a UV-light-scanner. The photos were printed out for evaluation of the amount of DNA-product in each sample. The bands created by the DNA was compared to the bands created by the ladder.

2.2.7 Purification and measurement

Purification of the samples was done using the reagent AMPure and its manual (Beckman Coulter Inc.) and using the bead-solution ratio 1:1,8, by eluting in 60 µL elution buffer from the Machery-Nagel DNA extraction kit. A Quantus™ Fluorometer was used to measure the DNA yield from the PCR (Life Technologies). Finally, concentrations were measured with Qubit Concentration measurement according to its manual.

The samples where after this step pooled together. For practical reasons the samples were divided on two pools. 36 of the samples from retention tree transects and 60 samples from forest edge samples were pooled together in pool 1 and 39 of the samples from retention tree transects and 60 samples from forest edge samples were pooled together in pool 2. The concentrations obtained by the Qubit Concentration measurement were used to calculate the volume needed to add an equal molar concentration of DNA from each sample to the pools. The samples E.Z.N.A.® Cycle Pure Kit was used to further clean up the pools from primers, nucleotides enzymes, salts, and other impurities. A pool quality check was done by using a 2100 Bioanalyzer instrument from Agilent.

2.2.8 Sequencing

The 120 samples from forest edge transects and 75 of the samples from the retention tree transects were sent to SciLifeLab in Sundsvall for sequencing. All of the samples could not fit in this round of sequencing, which is why only a fraction of the retention tree samples were sent.

2.3 Analysing the sequenced data

2.3.1 Taxa identifications

The sequence-data was as a start analysed using SCATA, which is a program that filter and cluster uploaded sequences and provide matching taxa names (Durling et al. 2011). The following settings were used: clustering distance: 0,012, minimum alignment to consider clustering: 0.99, mismatch penalty: 1, gap open penalty: 0, gap extension penalty: 1, end gap weight: 0, collapse homopolymers:3, downsample sample size:0, remove low frequency genotypes:1, tag-by-cluster max: 10000000, blast E-value cutoff: 1e-60, cluster engine: usearch, number of repseqs to report:3. To approve the sequence to belong to a certain species, the score had to be about 400, and identification at least 98,5%. The following thresholds were used as guidelines to determine taxonomic levels: 94.3%, 88.5%, 81.2% and 80.9% for in order genus, family, order, and class. If a sequence had an

identification value close to a threshold value, but not quite reaching up to it, I also took into consideration the likeliness for that being a correct identification, by taking into the account the number of similar matches, and if they came from type material or not. If there was no match, the sequence was additionally run against GeneBank (Sayers et al. 2022 and PlutoF Abarenkov et al. 2010), which are yet other programs that compare input sequences to sequences belonging to existing taxa. In GeneBank the search was limited to sequences from type material and optimized for somewhat similar sequences. The following settings were used in PlutoF: clustering engine: usearch, minimum alignment length for clustering: 0.99, maximum distance: 0.012000, mismatch penalty: 1.000000, gap open penalty: 0.000000, gap extension penalty: 1.000000, end gap weight: 0.000000, homopolymer reduction at: 3. The best match from either of these databases were then used as species hypothesis.

2.3.2 Sorting out ECM species

When a taxa hypothesis had been given to all the OTUs, a new file was created, only including taxa at genus level or higher. The species site matrix was divided on two separate datasets, one for forest edge-samples, one for retention tree-samples. When all OTUs at genus or species level was determined as ECM or not, the proportion of ECM species compared to all fungi sequences was calculated for all samples. Then, in the site-species matrix, all OTUs constituting less than 1% of each sample were removed. This was done to minimise to by chance including sequences of spores from fungi not established in the area. The ECM-community was as a start investigated by calculating the proportion of samples each species was present in compared to the total number of samples. Frequencies of species was also calculated for forest edge-samples and retention tree-samples separately.

2.3.3 Statistical analyses

All analyses from here on were done in R Studio, version 4.2.0 (RStudio Team, 2019). The two species matrixes were rarefied to a sequence depth of 289 for forest edge and 369 for retention trees. An appropriate sequence depth for each of the datasets was determined by having a look on the numbers of reads per sample, and by plotting a line of these with the number of reads on the y-axis and the number of samples sorted from least to the greatest number of reads on the x-axis. The sequence depth was chosen from where the rise of the line started to be less pronounced.

Linear model of ECM species richness and proportion of ECM species

The relative species richness of ECM between samples at different distances was analysed using the rarefied dataset, and the numbers of ECM species as response variable in linear mixed model using the function `lme()` from the package `nlme` (Pinheiro et al. 2022). Site was included as a random effect variable. With the same method, a linear model was used to analyse the relation between the proportion of ECM-reads and distance. The models were applied to both forest edge-samples and retention tree- samples. Any outliers were removed prior to running of the models.

ECM species composition

The two, non-rarefied, species matrixes were now normalized by transforming into binary data using the `decostand()` function from the `vegan` package (Oksanen 2022). The difference in species composition between samples, and possible differentiation regarding species composition between sites was visualised in a `nmDS`- plot. The `mds` and the plot was created with the functions `metaMDS()` with the following settings: `try=20`, `trymax=200`, `maxit=1000`, `noshare=T`, and `k=2`, and `ordiplot()`, both in the package `vegan()`. Statistically the same was assessed by performing a PERMANOVA (Anderson, 2001) using the function `adonis2()`, which also can be found in the `vegan`- package. Bray-curtis was used as a distance index, and the numbers of iterations was set to 9999. A post-hoc analysis was carried out using the functions `bcdist()`, which can be found in the package `ecodist()` (Goslee & Urban 2022) and `pairwise.perm.manova()` which can be found in the package `RVAideMemoire()` (Hervé 2022). Bray-curtis was used as a distance index, and the numbers of iterations was set to 9999 for the pairwise comparisons as well. A PERMDISP2-analysis (Anderson 2006, Anderson et al. 2006) was carried out to investigate wethere the difference in the species composition between distance-kategories could be explained by differing centroids, or if the difference could be found in the unequal spread of the distance-kategories.

3. Results

After quality control, and clustering of reads in SCATA 145684 reads were left (49 % of the total number of reads). In SCATA 1328 OTUs were identified, and of these 559 had a species hypothesis. After the remaining OTUs had been run in GeneBank and PlutoF, in total 1279 OTUs were identified as fungi. A total of 146 of these OTUs were identified as ECM. Several of the OTUs had the same species-hypothesis. After these were summed together, a total of 137 ECM-taxa remained. Of the 137 ECM-taxa 92 were identified to species level, and the remaining were identified to genus level. For the summarizing taxon/tables, and frequency/plots (Fig 14, Table 2-4), I hereon refer to these 137 ECM-taxa. In all other analyses however, I have used the original 146 OTUs. There were on average 806 (100-5927) reads belonging to fungi in each sample. Pool 1 had on average 445 (100-2623) and pool 2 had on average 586 (114-5927) reads belonging to fungi in each sample. The mean number of ECM reads were 111 for samples in the old growth forest, and: 218, 159, 111, 182, and 157 for samples in order 1, 3, 7, 15 and 30 m from the forest edge. The mean number of ECM reads were 440, 228, 40, 62,32 for samples in order 1, 3, 7, 15 and 30 m from the retention trees.

3.1 Species richness and proportion of ECM-reads in relation to distance

3.1.1 Forest edge

There is a negative correlation between distance and the number of ECM species ($f = 32.65469$, $p = <.0001$). This trend is visible in the boxplot of the mean sums of species for all distances and sites (Figure 7).

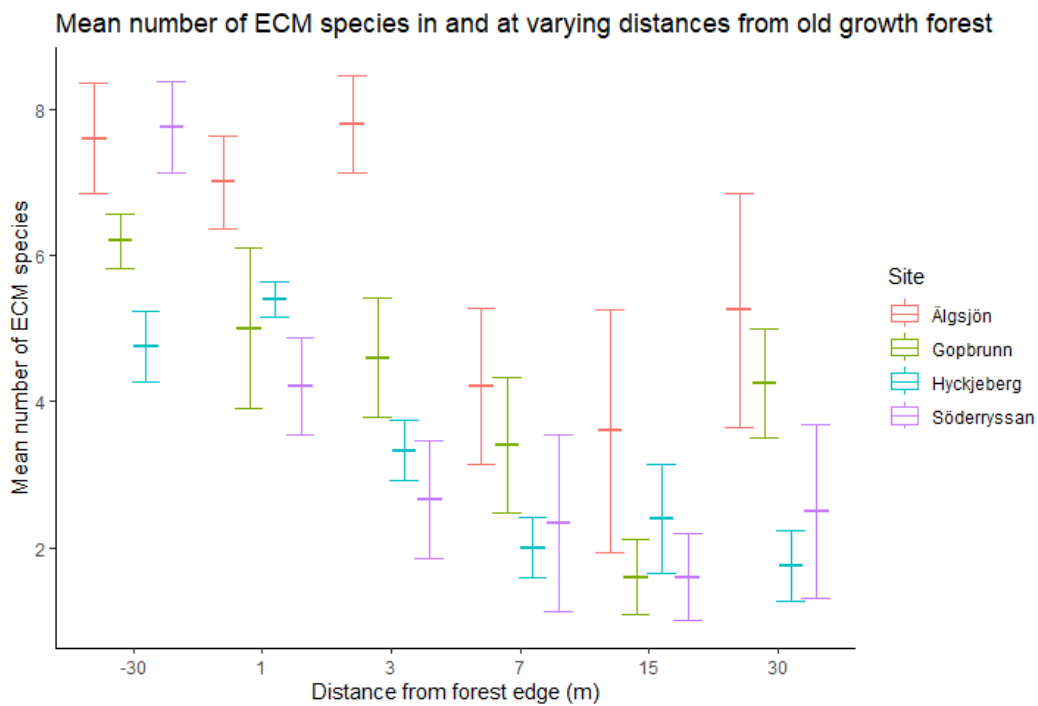


Figure 7 Mean number of ectomycorrhizal fungi and standard error at different distances into a clear cut area from the forest edge. The distance -30 m represents samples taken in old growth Scots pine forest. Samples are from four different clearcuts of old growth Scots pine-forest. There are 120 samples in total, 30 at each site, 20 samples for each distance category.

There is no correlation between distance and the proportion of ECM reads ($f = 0.14185$, $p = 0.7072$). This is illustrated in a boxplot of the mean proportion of ECM species for all distances and sites. The boxplot indicates that this trend is true for all sites (Figure 8).

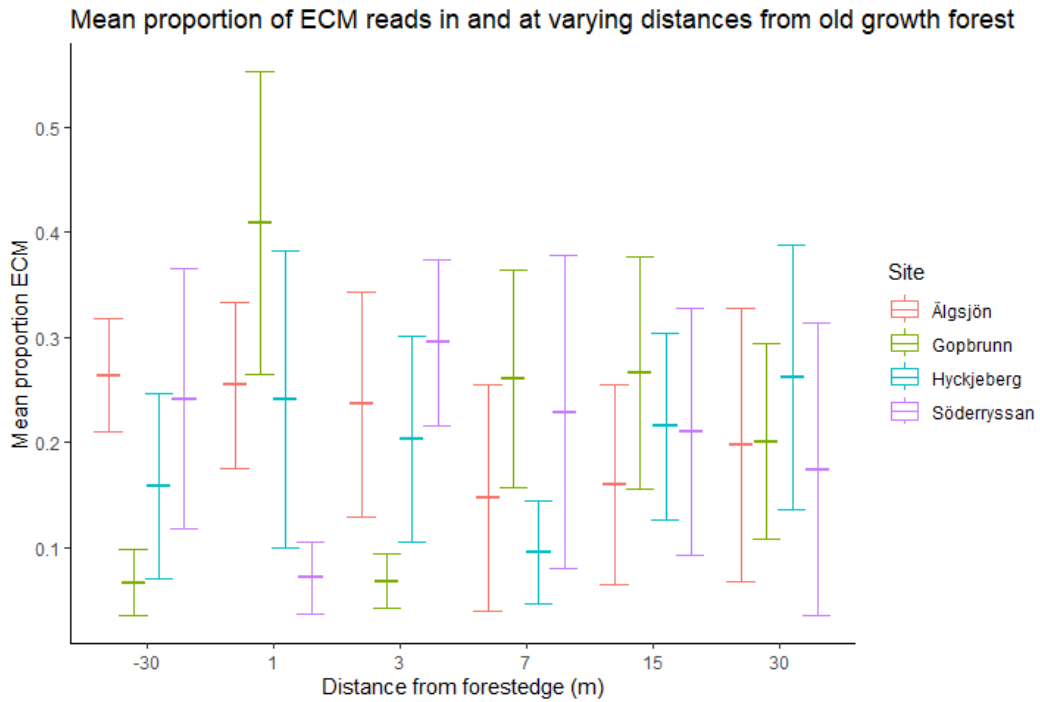


Figure 8 The mean proportion of ECM-reads, compared to all reads in each sample, and standard error, at different distances into a clear cut area from the forest edge. The distance -30 m represents samples taken in old growth Scots pine forest. Samples are from four different clearcuts of old growth Scots pine-forest. There are 120 samples in total, 30 at each site, 20 samples for each distance category.

3.1.2 Retention trees

There is a correlation between distance from the retention trees and the number of ECM species ($f = 5.03990$, $p = 0.028$). This trend is visible in the boxplot of the mean sums of species for all distances and sites (Figure 9).

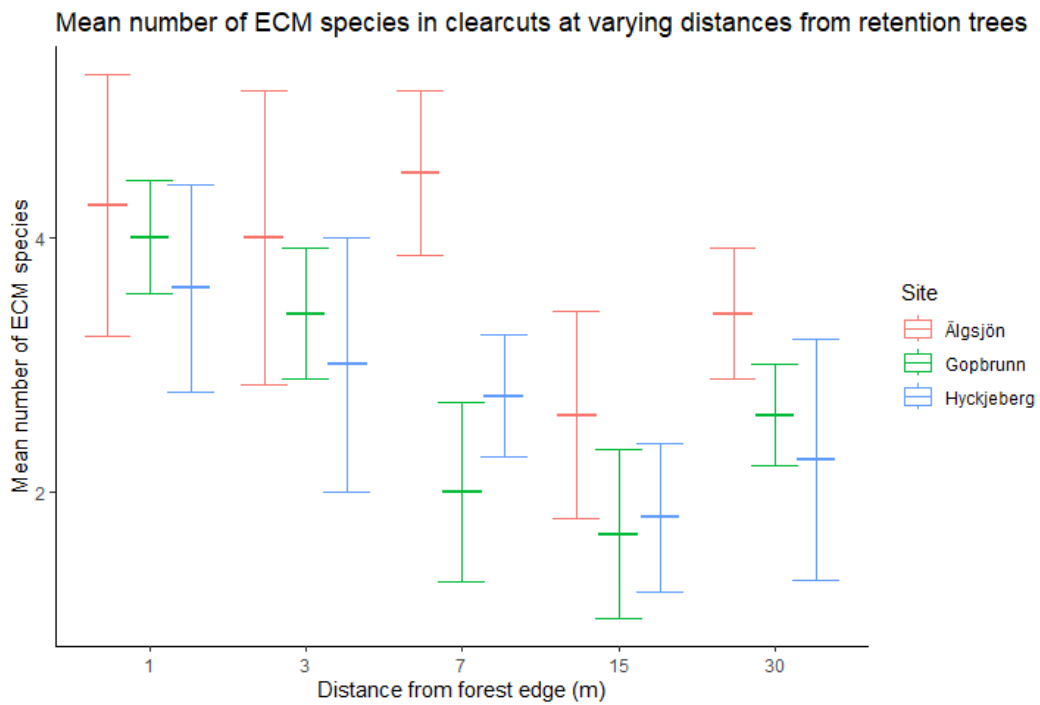


Figure 9 The mean number of ECM species, and standard error, at different distances from single retention trees. Samples are from three different clearcuts of old growth Scots pine-forest. There are 75 samples in total, 25 at each site, 15 samples for each distance category.

There is a negative correlation between distance and the proportion of ECM reads ($f = 21.62769$, $p = <.0001$). This trend is visible in the boxplot of the mean proportion of ECM species for all distances and sites. The boxplot indicates that this trend is true for all sites (Figure 10).

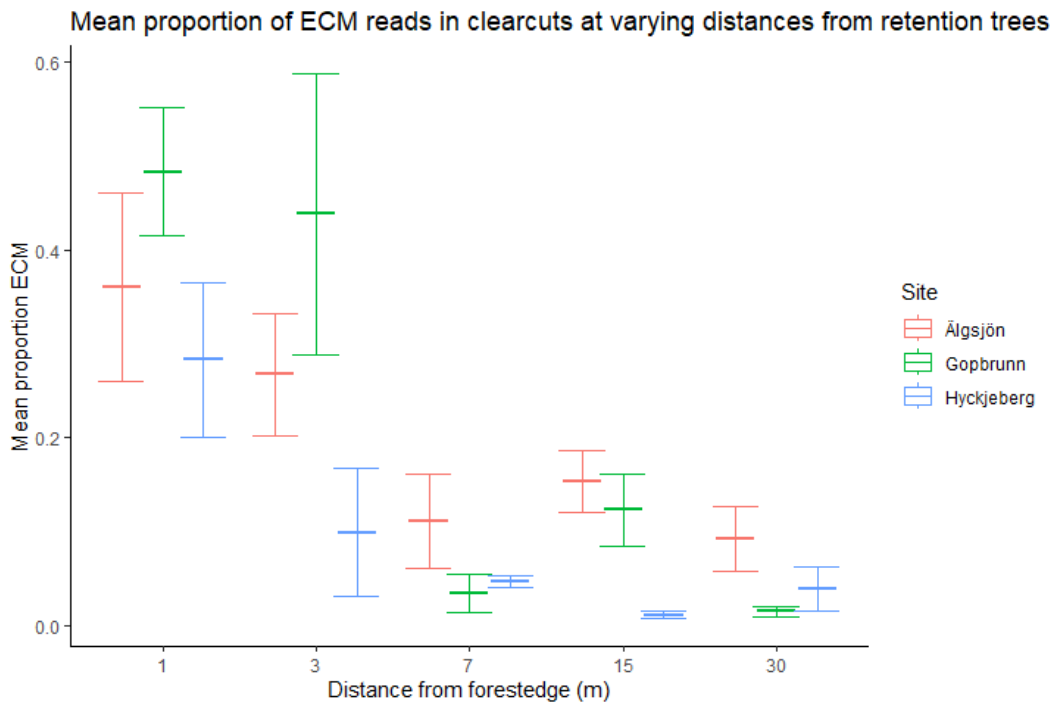


Figure 10 The mean proportion of ECM-reads compared to the total number of reads in each sample, and standard error, at different distances from single retention trees. Samples are from tree different clearcuts of old growth Scots pine-forest. There are 75 samples in total, 25 at each site, 15 samples for each distance category.

3.2 ECM-community composition in relation to distance

3.2.1 Forest edge

The PERMANOVA for forest edge-samples is significant ($f=5.4664$, $p=1e-04$), indicating differences in species composition among samples taken at varying distances from the forest edge. A PERMDISP2-analysis on the same data does not give a significant result ($f=2.0838$, $p=0.07301$), which states that the difference in species composition between sites does not come from the difference in the spread

of samples in the multivariate analysis, but rather that the centroids, and thereby the species composition, differs between the distance-categories.

A post-hoc test with pairwise corrections with Bonferroni-Holm corrections, gives that forest samples differs significantly from samples at 7 ($p=0.0052$), 15 ($p=0.0015$), and 30 ($p=0.0015$) meters distance. Samples at 1 compared to 15 meters distance from the forest edge also differs significantly from each other ($p=0.0072$). No other pairwise comparisons are significantly different from each other. The Bonferroni-Holm-corrected pairwise comparisons for the PERMDISP2 gave no significant result for any of the compared distances.

The above-described results are visualized in a nMDS-plot in Figure 12. With the number of dimensions = 2, there is a stress level of 0.2336055.

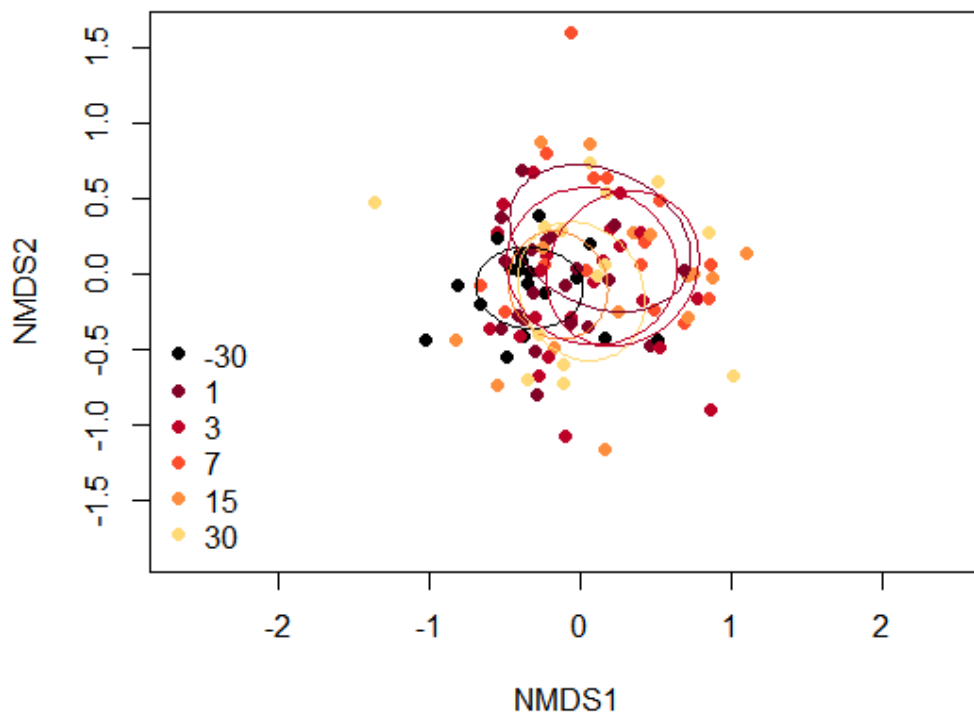


Figure 11 An nMDS-plot visualizing the similarity of ECM fungal community composition among samples along the transect from forest into clearcuts. Samples from all sites are included. The samples are coloured coded for different distances in meter from the forest edge. -30 represents the samples taken in the forest. The stress of the nMDS is 0.2336055.

3.2.2 Retention trees

The PERMANOVA for retention tree-samples is significant ($f= 3.1047$, $p=0.0031$), indicating differences in species composition among samples taken at varying distances from retention trees. A PERMDISP2-analysis on the same data does not give a significant result ($f= 1.0592$, $p= 0.3846$) which states that the difference in species composition between sites does not come from the difference in the spread of samples in the multivariate analysis, but rather that the centroids, and thereby the species composition differs between the distance-categories.

A post-hoc test with pairwise corrections with Bonferroni-Holm corrections, gives that the following distances differs significantly from each other regarding species composition 3 m:30 m ($p=0.024$), all other pairwise comparisons are not significantly different from each other. The Bonferroni-Holm-corrected pairwise comparisons for the PERMDISP2 gave no significant result for any of the compared distances.

The above-described results are visualized in a nMDS-plot in Figure 13. With the number of dimensions = 2, there is a stress level of 0.2253835.

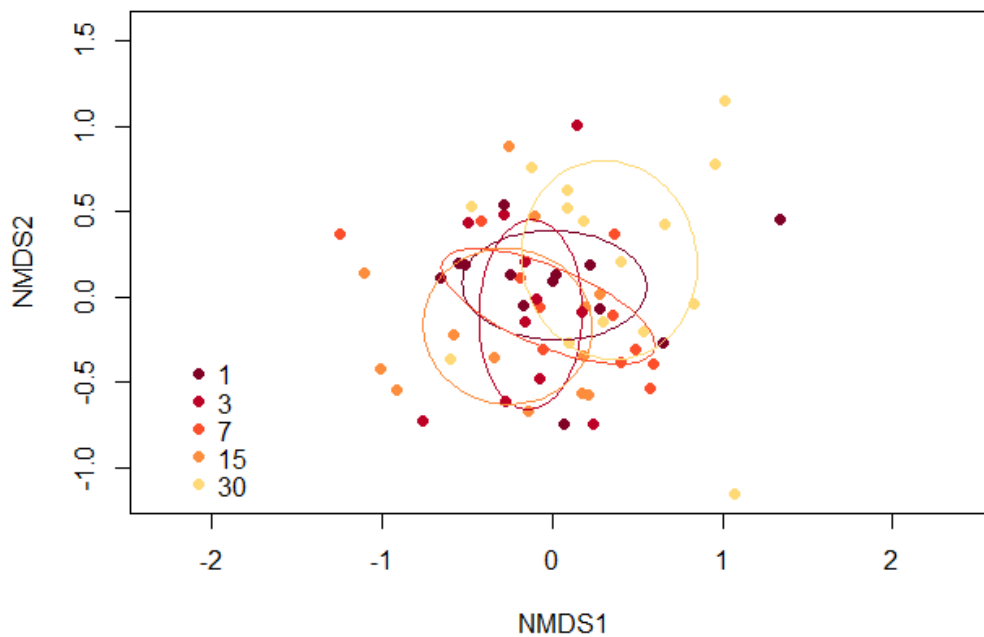


Figure 12 An nMDS-plot visualizing the similarity of ECM fungal community composition among samples along the transect from retention trees forest into clearcuts. Samples from all sites are included. The samples are coloured coded for different distances in meter from the retention trees. The stress of the nMDS is 0.2253835.

3.3 Overall observed ECM-community structure

When looking at all 195 samples, both forest edge- and retention tree- samples, the most species-rich and abundant genera were *Cortinarius* (30 species, 33,4% of reads), *Russula* (14, 27,2%) *Lactarius* (6, 2%), *Hydnellum* (5, 1,2%), *Piloderma* (4, 12,1%), *Suillus* (3, 6,7%), *Elaphomyces* (3, 1,8%), and *Tomentellopsis* (3, 0,2%). *Russula decolorans* was the species that had the highest relative abundance, with 22 % of the total number of ECM reads. The second most abundant species *Cortinarius caperatus*, representing 19 % of the total number of reads. *Piloderma sphaerosporum* was the third most abundant species, with 10% of the total number of reads. The majority of the ECM species had low relative abundances.

Three species were recorded in 43% of the samples or more: *Cenococcum geophilum* (coll.) (58%), *Piloderma sphaerosporum* (49%), and *Russula decolorans* (43%). Most species however had a low frequency, they only occurred in a few samples (Fig 15, Table 2).

By looking at the number of ECM species found in each distance-category (forest edge-samples), more ECM species were found in the control-samples in the old growth forest than on the clearcut, and the number of species decline with the distance from the forest edge. The same applies to retention trees, where the raw number of species is higher for samples closer to the tree. In the old growth forest, 70 species were found. In samples 1 m from the forest edge; 73 species were found, 67 species at 3 m, 39 species at 7 m, 29 species at 15 m and 35 species at 30 m. At 1 m from the retention trees 40 species were found, and 39 species at 3 m, 31 species at 7 m, 19 species at 15 m and 30 species at 30 m. When comparing samples from the old growth forest with samples 30 m from the forest edge, there was a decline in species number from 70 to 35 (table 3). When comparing samples 1 m to those 30 m from the retention trees, there was a decline in species number from 40 to 30, (table 4). The two genera with the most pronounced decline in species numbers when comparing old growth forest with samples 30 m from the forest edge, is *Cortinarius* and *Russula*, which also are the most species rich genera. They decline from 24 to 7 and 10 to 5 ECM species respectively. The same pattern can be seen for retention trees where *Cortinarius* and *Russula*, which decline from 10 to 5 and 7 to 4 ECM species respectively. No genera increase in species number with increasing distance to forest edge or retention trees.

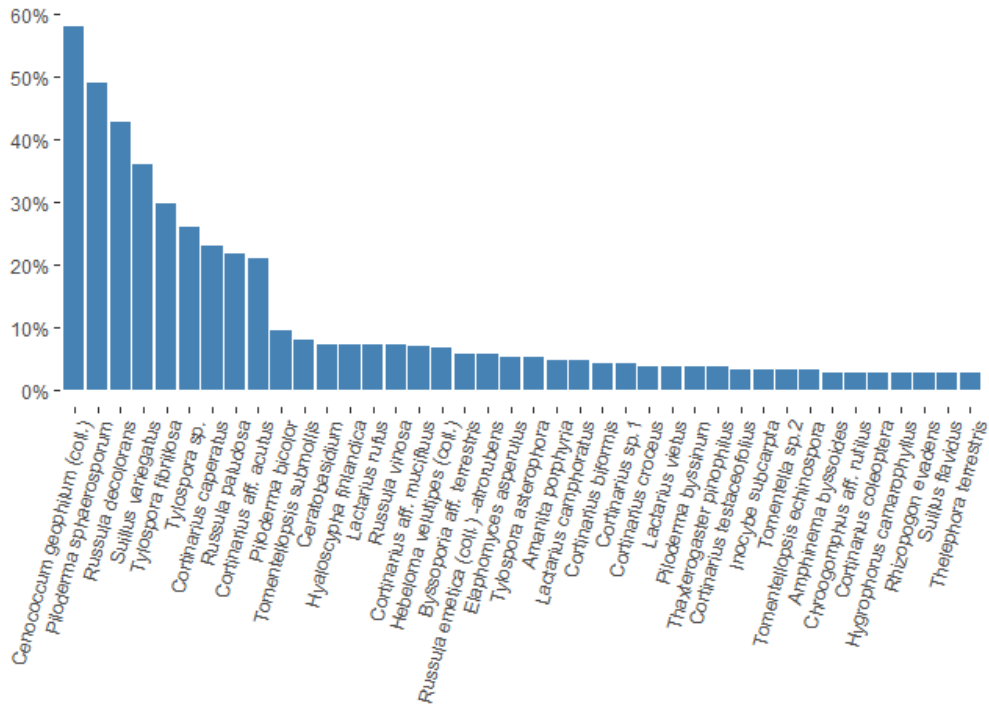


Figure 13 The top 40 most frequent ECM species that was found in the study. All samples from both forest edge-transects, and retention tree-transects are here summed together, to a total of 192 samples. The y-axis represents the % of all samples.

Table 2. The table shows the top 20 most frequent ECM species for all samples. In total there are 195 samples. The column with the title Nr. samples, shows the total number of samples of all 195 samples, in which a species occurs. Frequency equals the percentage of the total number of samples a species occurs in. The full table can be found in Appendix 1

Species name	Redlist	Nr. samples	Frequency
<i>Cenococcum geophilum</i> (coll.)	LC	112	58,0
<i>Piloderma sphaerosporum</i>	LC	94	49,0
<i>Russula decolorans</i>	LC	82	42,7
<i>Suillus variegatus</i>	LC	69	35,9
<i>Tylospora fibrillosa</i>	LC	57	29,7
<i>Tylospora sp.</i>	LC	50	26,0
<i>Cortinarius caperatus</i>	LC	44	22,9
<i>Russula paludosa</i>	LC	42	21,9
<i>Cortinarius aff. acutus</i>	LC	41	21,0
<i>Piloderma olivaceum</i>	LC	32	17,0
<i>Cortinarius semisanguineus</i>	LC	26	13,5
<i>Cortinarius aff. obtusus</i>	LC	22	11,5

<i>Piloderma bicolor</i>	LC	18	9,4
<i>Tomentellopsis submollis</i>	LC	16	8,0
<i>Russula vinosa</i>	LC	14	7,3
<i>Lactarius rufus</i>	LC	14	7,3
<i>Hyaloscypha finlandica</i>	LC	14	7,3
<i>Ceratobasidium</i>	LC	14	7,3
<i>Cortinarius aff. mucifluus</i>	LC	14	7,0
<i>Hebeloma velutipes (coll.)</i>	LC	13	6,8

Table 3. The table shows the top 20 most frequent ECM species for samples taken along forest edges. There are 120 samples in total, 30 at each site, 20 samples for each distance category. The table lists the numbers of occurrences at different distances from forest edge. The column with the title "total" shows the sum of occurrences for each species. The full table can be found in Appendix 1.

Species name	-30	1	3	7	15	30	Total
<i>Cenococcum geophilum (coll.)</i>	15	13	15	11	8	11	73
<i>Piloderma sphaerosporum</i>	16	16	15	4	3	3	62
<i>Suillus variegatus</i>	17	14	12	4	2	2	50
<i>Russula decolorans</i>	4	8	9	9	10	10	45
<i>Tylospora sp.</i>	7	9	10	4	2	2	35
<i>Tylospora fibrillose</i>	5	8	8	3	3	3	31
<i>Cortinarius aff. acutus</i>	11	7	7	2	1	1	28
<i>Cortinarius caperatus</i>	5	7	7	2	3	3	27
<i>Piloderma olivaceum</i>	11	6	4	0	1	1	24
<i>Russula paludosa</i>	2	2	3	5	4	4	21
<i>Cortinarius semisanguineus</i>	4	6	5	1	4	4	20
<i>Cortinarius aff. obtusus</i>	3	5	4	2	2	2	17
<i>Piloderma bicolor</i>	6	3	1	1	0	0	12
<i>Tomentellopsis submollis</i>	1	2	6	1	0	0	11
<i>Lactarius rufus</i>	2	3	4	1	1	1	11
<i>Hyaloscypha finlandica</i>	2	1	1	3	2	2	11
<i>Hebeloma velutipes (coll.)</i>	3	6	1	0	0	0	10
<i>Ceratobasidium</i>	1	1	3	2	0	0	9
<i>Cortinarius aff. mucifluus</i>	4	0	2	0	2	2	8
<i>Tylospora asterophora</i>	1	4	1	0	2	2	8

Table 4. The table shows the top 20 most frequent ECM species for samples taken around retention trees. There are 120 samples in total, 30 at each site, 20 samples for each distance category. The table lists the numbers of occurrences at different distances from the retention trees. The column with the title "total" shows the sum of occurrences for each species. The full table can be found in Appendix 1.

Species name	1	3	7	15	30	Total
<i>Cenococcum geophilum (coll.)</i>	12	7	5	5	10	39
<i>Russula decolorans</i>	10	9	8	7	3	37
<i>Piloderma sphaerosporum</i>	11	10	3	4	4	32
<i>Tylospora fibrillose</i>	6	5	7	2	6	26
<i>Russula paludosa</i>	4	6	3	6	2	21
<i>Suillus variegatus</i>	8	9	1	0	1	19
<i>Cortinarius caperatus</i>	5	5	4	2	1	17
<i>Tylospora sp.</i>	7	2	1	2	3	15
<i>Cortinarius aff. acutus</i>	8	3	0	2	0	13
<i>Piloderma olivaceum</i>	4	2	2	0	0	8
<i>Russula vinosa</i>	2	0	2	0	3	7
<i>Cortinarius semisanguineus</i>	3	1	0	1	1	6
<i>Piloderma bicolor</i>	1	1	2	1	1	6
<i>Elaphomyces asperulus</i>	1	2	0	2	1	6
<i>Cortinarius aff. obtusus</i>	3	2	0	0	0	5
<i>Tomentellopsis submollis</i>	2	2	1	0	0	5
<i>Ceratobasidium</i>	0	1	1	1	2	5
<i>Byssoporia aff. terrestris</i>	1	2	1	0	1	5
<i>Russula emetica (coll.) -atrorubens</i>	2	1	1	0	0	4
<i>Lactarius camphoratus</i>	2	2	0	0	0	4

3.4 Red-listed species

In total, looking at all 195 samples, five red-listed species were found: *Hydnellum gracilipes* (VU), *Phellodon niger* (NT), *Elaphomyces leveillei* (NT), *Hydnellum caeruleum* (NT), *Hydnellum aurantiacum* (NT). *Hydnellum gracilipes* was found in one sample in the site Hyckjeberg at 1 m from the forest edge. *Phellodon niger* was found in one sample in Älgsjön, one in Hyckeberg and two in Söderryssan, at distances 1 m (3) and 7 m (1) from the forest edge. *Elaphomyces leveillei* was found in two samples in Söderryssan, both in the old growth forest. *Hydnellum caeruleum* was found in one sample in the location Älgsjön at 1 m from the forest edge. *Hydnellum aurantiacum* was found in one sample in the location Älgsjön at 3 m from a retentientree.

4. Discussion

This study had the goal to gain knowledge on how the ectomycorrhizal community (composition and richness) change along transects from old growth Scots pine forest to a gradually increasing distance out on clearcuts of the same forest, and how this change along transects from retention trees on these clearcuts. With a total of 192 samples, distributed on 4 sites, 137 species of ECM were found. The study shows that the number of ECM species is 50% lower 30 m out on 1–2-year-old clearcuts of old growth pine forest, compared to intact old growth pine forest, and that the number of ECM species decline along a 30 m long gradient from the old growth forest. The study observed no change in the proportion of ECM reads compared to all fungal reads along a 30 m long gradient from the old growth forest. The study shows that the number of ECM species is 25% lower 30 m out on 1–2-year-old clearcuts of old growth pine forest, compared to 1 m around single retention trees. and that the number of ECM species decline along a 30 m long gradient from the retention trees. The study additionally observed a change in the proportion of ECM reads compared to all fungal reads along the transects around the retention trees.

The ECM species composition also changes significantly with the distance to the forest edge. The species composition at 1 and 3 m from the forest edge, were similar with the species composition in the old growth forest, whereas it differed significantly at 7m, 15m, and 30m. The ECM species composition changed significantly with distances from the retention trees. However, pairwise comparisons shows that only the species composition at 3 m from the retention trees differed significantly from the species composition at 30 m from the retention trees. These findings are in line with my hypothesis that the ECM species number would decline with increasing distance from forest edges and retention trees. My finding that the proportion of ECM reads declines with increasing distance from retention trees are also in line with my hypothesis. However the proportion of ECM reads was not affected by the distance from the forest edge, this result was the opposite from what I hypothesized.

4.1 ECM species richness and distance

The trend where the number of species decline with increasing distance from the forest edge is in line with previous findings of the species richness being higher in old forest compared to clearcuts (Wallander et al. 2010, Sterkenburg et al. 2019). That a change could be seen in the number of ECM species with the distance to

retention trees is also in line with previous studies (Luoma et al. 2006). As me, Louma found that there was a negative correlation with distance and the number of ECM species. Even though the ECM species numbers declined, there were ECM species present on the clearcut, which is interesting, because ECM species cannot survive without their host trees, and therefore I expected not be able to find any ECM fungi on the clearcut, if there are no living trees. As pine roots typically may reach about 10 meters, less frequently further, in theory there should be a lower number of species at larger distances, as a higher density of roots is correlated with number of species (Kalliokoski et al. 2008, Djupström 2022). My theory why I observed ECM species 15 meters and further out on the clearcut is that small seedlings were left on the clearcut from before the felling at all sites. At closer distances the density of roots from grown up trees should be higher than the density of roots from small seedlings, and therefore play the main role in the number of ECM species. Another possibility is that some of the species were sustained by a few far-reaching roots. If this is the case, this has an influence on the results for species richness for both forest edge and retention trees.

It is unlikely with surviving mycelia of ectomycorrhizal fungi after two years in the absence of living tree roots. The mycorrhizal contribution to soil respiration is reported to decline with 54% within 1-2 months after their host trees has died (Högberg et al. 2001). One aspect to keep in mind, is that my study only included a relatively small number of samples, and that the beta diversity for ECM is high. By chance, the number of species numbers vary.

The notation that the number of species in the genus *Cortinarius* decline with increasing distance to forest edge and retention trees have been observed in several other studies (Wallander et al. 2010, Kyaschenko et al. 2017, Lindahl et.al 2021, Djupström 2022). Species from the genus *Pilodema* have previously been observed to be less sensitive to logging, in my study, this genus declines in number of species 5 to 3 for forest edge samples and 4 to 2 for retention tree-samples (Kyaschenko et al. 2017).

4.2 ECM-community composition and distance

The changes in ECM species composition with distance from the forest edge is consistent with previous studies. For example, a study by Peay et al. (2012) found that ECM species richness declined, and community composition changed with increasing distance from the edge of a California old-growth forest. My results that the species composition change at the distance 7 m is in line with previous studies, which repeatedly have observed that the mycorrhiza-mycelia mostly occur withing

5-6 meters from their host trees (Djupdal et al. 2022, Saari et al. 2005, Göttlicher et al. 2008) and that the species composition also changes at this distance (Luoma et al. 2006). The community in the clearcut is mostly composed of the same species that are also present on the forest, but not the opposite. My interpretation of the significant difference in ECM community composition between samples at 3 and 30 m from the retention trees, and lack of a significant result for all other distance comparisons, is that the retention trees mostly are colonized by the same species that are also present on the clearcut. This result contradicts a previous observation that an ECM-community 1 m from single retention trees differs from that in a harvested area further away from any retention trees (Varenius et al. 2016).

As can be seen in the results of this study, some more species appeared to survive associated to retention trees, since there were a larger number of species close to the trees and a larger proportion of the fungal reads belonged to ECM species compared to further away. The decline in the numbers of species (25%) along the retention tree-transects, indicate that retention trees sustain more species compared to clearcut areas without retention trees. This reasoning holds at least if we assume that there are no pine roots at the distance 30 m from the retention trees, and if we view those samples as existing on a bare clearcut. As pine roots typically reach about 10 m, rarely longer (Kalliokoski et al. 2008), it should be ok to make this assumption. The more trees that are left per unit area, the higher is the number of roots per unit area. As the density of fine roots, is a driving factor for the abundance and species richness of ECM, the species richness will be higher the more trees that are left (Peay et al. 2011).

Retention trees can obviously sustain a part of an ECM community from forest prior to cutting, and harbour a larger proportion of fungi DNA per soil unit belonging ECM species compared to a bare clearcutting. This holds true at least for frequent species. The more trees that are removed from a forest, the higher is the risk that rare species disappear by chance (Sterkenburg et al. 2019). This comes as a result of the general fungi community structure, where a few species dominate, whereas most of the species are rare (Horton & Bruns 2001). How well retention trees could be at sustaining infrequent species is a tricky question to answer other than theoretically, since it is only for the more frequent species such statistics can be calculated.

4.3 Overall observed ECM-community structure

The total of 137 ECM species obtained by collecting in total 192 samples, is similar to the number of species found in the same habitat with a similar sampling effort.

In another study of *Pinus sylvestris*, 149 species was found when collecting in total 180 samples (Sterkenburg et al 2019). And in yet another study, also in *Pinus sylvestris* habitat, 141 ECM species were found when 368 samples were analysed (Djupström et al. 2022).

For this study, the most species-rich and abundant genera were (in order): *Cortinarius*, *Russula*, *Lactarius*, *Hydnellum*, *Piloderma*, *Suillus*, and *Elaphomyces*. This pattern largely corroborates with the community pattern that has previously been observed for this habitat. The exception is *Hydnellum* and *Elaphomyces*, instead *Inocybe* and *Tomentella* have previously been observed among the most species-rich and abundant genera (Sterkenburg et al. 2019).

Due to the few numbers of sites, four, it is not possible to calculate statistics of how the frequency of species and genera may be affected with increasing distance to forest edges or retention trees. However, when looking at the frequencies of all samples from all sites and transects combined for each distance, we get 20 samples in total. By looking at the topmost frequent species with this method of calculation, it appears that they occur similarly frequent in forests as at different distances and around retention trees. *Russula decolorans* is an exception, this species appear to increase its frequency in clearcuts.

4.4 Red-listed species

Red-listed species were only found close to forest edge and retention trees. These findings imply that these red-listed species are sensitive to clearcutting. I cannot completely rule out that any of the sequences came from spores from fungi not established in the area as mycelia. In the extreme case, this would mean that these red-listed species do not occur in my sample area at all, not in the old growth forest, nor in the clearcut, but merely that they are present as spores. I however do not find this likely. If I for example check the number of reads of *Phellodon niger* in the samples where this species occurs, I get that 75 out of 353 reads belong to this species in one of the samples, and in another of the samples 11 out of 1968 reads. My interpretation is that I, at least in some of the cases, have too many reads of this species for it to be likely that the sequences origins exclusively from spores. I take this reasoning from the knowledge that 95% of the spores end up within a few meters from the sporocarp, and thus it is not likely to have high densities of spores far away from a mycelium producing sporocarps. That I found two red-listed species in samples around the retention trees suggests that these may act as lifeboats for a few individuals of some red-listed species. Red-listed species that also are rare, are more likely to disappear by chance, the more trees that are removed from the forest.

4.5 Conclusion

In conclusion, this study provides insights into how clearcutting and the leaving of retention trees affects ECM species diversity and ECM species composition. The observed declines in ECM species numbers with increasing distance from the forest edge, illustrates that clearcutting can have negative effects on ECM biodiversity if a to large proportion of the forests undergo clearcutting.

The negative correlation between ECM species numbers and increasing distance from single retention trees suggest that single retention trees can have a small effect in increasing the total diversity of ECM species, although the lack of affect of distance on the community structure, show that the species are shared between forests and the clearcut. Single retention trees may lifeboat the ECM species that were associated with them before the clearcutting event, but most of these species will be frequent species, whereas it is less likely that infrequent species will be lifeboatated by single trees. An interesting finding is the number of ECM species that were present on the clearcut. Many of these were associated with pine roots that reached out on the clearcut. In addition, some may be associated with small seedlings that remained from before the forest were felled. As the density of roots is higher closer to the forest edge, the roots from mature trees likely plays the major role to the occurrence of ECM closer to the forest edge, while small seedlings should be of an increasing importance further away as the density of roots from mature trees decrease.

The observed change in community composition at increasing distance from forest edge corrode with observations from other studies. These findings show that clearcutting has a negative impact on ECM diversity associated with old growth forest. These findings implies that preservation of ECM diversity associated with later stages of forest succession require a higher level of forest tree continuity than clear-cut management within forest management of today results in.

References

- Abarenkov, Abarenkov, K., Tedersoo, L., Nilsson, R. H., Vellak, K., Saar, I., Veldre, V., Parmasto, E., Prous, M., Aan, A., Ots, M., Kurina, O., Ostonen, I., Jõgeva, J., Halapuu, S., Põldmaa, K., Toots, M., Truu, J., Larsson, K., Kõljalg, U. 2010. PlutoF - a Web Based Workbench for Ecological and Taxonomic Research, with an Online Implementation for Fungal ITS Sequences. *Evolutionary Bioinformatics* 6, 189 – 196.
- Ahlström, A., Canadell, J.G., Metcalfe, D.B., 2022. Widespread Unquantified Conversion of Old Boreal Forests to Plantations. *Earth's Future* 10, e2022EF003221. <https://doi.org/10.1029/2022EF003221>
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46. <https://doi.org/10.1046/j.1442-9993.2001.01070.x>
- Anderson, M.J., 2006. Distance-Based Tests for Homogeneity of Multivariate Dispersions. *Biometrics* 62, 245–253. <https://doi.org/10.1111/j.1541-0420.2005.00440.x>
- Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. *Ecology Letters* 9, 683–693. <https://doi.org/10.1111/j.1461-0248.2006.00926.x>
- Barker, J.S., Simard, S.W., Jones, M.D., Durall, D.M., 2013. Ectomycorrhizal fungal community assembly on regenerating Douglas-fir after wildfire and clearcut harvesting. *Oecologia* 172, 1179–1189. <https://doi.org/10.1007/s00442-012-2562-y>
- Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* 220, 1108–1115. <https://doi.org/10.1111/nph.14976>
- Dahlberg, A., Mueller, G.M., 2011. Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species. <https://doi.org/10.1016/j.funeco.2010.11.001>

- Djupström, L., Dahlberg, A., Lindahl, B., 2022. Nyttan av naturhänsyn för marksvampar. Resultat fem år efter avverkning. ARBETSRAPPORT 1116–2022. Skogforsk.
- Durling B. M., Clemmensen E. K., Stenlid J., Lindahl B. 2011. SCATA - An efficient bioinformatic pipeline for species identification and quantification after high-throughput sequencing of tagged amplicons (submitted).
- Bücking, H., Liepold, E., Ambilwade, P., Bücking, H., Liepold, E., Ambilwade, P., 2012. The Role of the Mycorrhizal Symbiosis in Nutrient Uptake of Plants and the Regulatory Mechanisms Underlying These Transport Processes, *Plant Science*. IntechOpen. <https://doi.org/10.5772/52570>
- Carteron, A., Beigas, M., Joly, S., Turner, B.L., Laliberté, E., 2021. Temperate Forests Dominated by Arbuscular or Ectomycorrhizal Fungi Are Characterized by Strong Shifts from Saprotrophic to Mycorrhizal Fungi with Increasing Soil Depth. *Microb Ecol* 82, 377–390. <https://doi.org/10.1007/s00248-020-01540-7>
- Cline, E.T., Ammirati, J.F., Edmonds, R.L., 2005. Does proximity to mature trees influence ectomycorrhizal fungus communities of Douglas-fir seedlings? *New Phytologist* 166, 993–1009. <https://doi.org/10.1111/j.1469-8137.2005.01387.x>
- Dahlberg, A., Schimmel, J., Taylor, A.F.S., Johannesson, H., 2001. Post-Fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. *Biological Conservation*.
- De la Varga, H., Águeda, B., Martínez-Peña, F., Parladé, J., Pera, J., 2012. Quantification of extraradical soil mycelium and ectomycorrhizas of *Boletus edulis* in a Scots pine forest with variable sporocarp productivity. *Mycorrhiza* 22, 59–68. <https://doi.org/10.1007/s00572-011-0382-2>
- Djupström, L., Dahlberg, A., Lindahl, B., 2022. Nyttan av naturhänsyn för marksvampar.
- Eide, G.I., 2020. Tillstånd och trender för arter och deras livsmiljöer – rödlistade arter i Sverige 2020 - SLU Artdatabanken.
- Figueiredo, L., Krauss, J., Steffan-Dewenter, I., Sarmiento Cabral, J., 2019. Understanding extinction debts: spatio-temporal scales, mechanisms and a roadmap for future research. *Ecography* 42, 1973–1990. <https://doi.org/10.1111/ecog.04740>
- Galante, T.E., Horton, T.R., Swaney, D.P., 2011. 95 % of basidiospores fall within 1 m of the cap: a field-and modeling-based study. *Mycologia* 103, 1175–1183. <https://doi.org/10.3852/10-388>

- Gehring, C. A., Theimer, T. C., & Whitham, T. G., 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. *Ecology*, 79(5), 1562-1572.
- Glassman, S.I., Lubetkin, K.C., Chung, J.A., Bruns, T.D., 2017. The theory of island biogeography applies to ectomycorrhizal fungi in subalpine tree “islands” at a fine scale. *Ecosphere* 8, e01677. <https://doi.org/10.1002/ecs2.1677>
- Goslee, S. C., & Urban, D. L., 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, 22(7), 1-19. doi: 10.18637/jss.v022.i07
- Göttlicher, S. G., Taylor, A.F.S., Grip, H., Betson, N.R., Valinger, E., Högberg, M.N., Högberg, P., 2008. The lateral spread of tree root systems in boreal forests: Estimates based on 15N uptake and distribution of sporocarps of ectomycorrhizal fungi. *Forest Ecology and Management* 255: 75-81.
- Hallingbäck, T. & Aronsson, G., 1998. *Ekologisk katalog över storsvampar och myxomyceter*. Andra, reviderade och utökade upplagan uppl. Uppsala: ArtDatabanken, SLU.
- Hawksworth, D.L., Lücking, R., 2017. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiol Spectr* 5. <https://doi.org/10.1128/microbiolspec.FUNK-0052-2016>
- Heinonsalo, J., Sun, H., Santalahti, M., Bäcklund, K., Hari, P., Pumpanen, J., 2015. Evidences on the Ability of Mycorrhizal Genus *Piloderma* to Use Organic Nitrogen and Deliver It to Scots Pine. *PLOS ONE* 10, e0131561. <https://doi.org/10.1371/journal.pone.0131561>
- Hervé, M., 2022. *RVAideMemoire: Testing and Plotting Procedures for Biostatistics* (Version 0.9-81-2). Retrieved from <https://CRAN.R-project.org/package=RVAideMemoire>
- Hoffland, E., Giesler, R., Jongmans, A.G., Breemen, N. van, 2003. Feldspar Tunneling by Fungi along Natural Productivity Gradients. *Ecosystems* 6, 739–746. <https://doi.org/10.1007/s10021-003-0191-3>
- Horton, T.R., Bruns, T.D., 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10, 1855–1871. <https://doi.org/10.1046/j.0962-1083.2001.01333.x>
- Horton, T. R., Bruns, T. D., 1998. Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed stand of Douglas-fir (*Pseudotsuga menziesii*) and bishop pine (*Pinus muricata*) *New Phytol.* **139**, 331–339.

- Hyvärinen, E., Juslén, A., Kemppainen, E., Uddström, A. Liukko, U., 2019. Suomen lajien uhanalaisuus – Punainen kirja 2019; The Red List of Finnish Species 2019. Ympäristöministeriö & Suomen ympäristökeskus.
- Högberg, P., Johannisson, C., Yarwood, S., Callesen, I., Näsholm, T., Myrold, D.D., Högberg, M.N., 2011. Recovery of ectomycorrhiza after ‘nitrogen saturation’ of a conifer forest. *New Phytologist* 189, 515–525. <https://doi.org/10.1111/j.1469-8137.2010.03485.x>
- Kalliokoski, T., Nygren, P., & Sievänen, R. (2008). Comparison of *Pinus sylvestris* and *Picea abies* fine root architecture in different forest soils. *Plant and Soil*, 304(1-2), 81-93.
- Kalliokoski, T., Pennanen, T., Nygren, P., Sievanen, R., Helmisaari, H.-S., 2010. Belowground interspecific competition in mixed boreal forests: fine root and ectomycorrhiza characteristics along stand developmental stage and soil fertility gradients. *Plant Soil* 330, 73–89. <https://doi.org/10.1007/s11104-009-0177-9>
- Kuuluvainen, T., 2009. Forest management and biodiversity conservation based on natural ecosystem dynamics in northern Europe: the complexity challenge. *AMBIO: A Journal of the Human Environment*, 38(6), 309-315. <https://doi.org/10.1579/08-A-490.1>
- Kuuluvainen, T., Tahvonen, O., Aakala, T., 2012. Even-Aged and Uneven-Aged Forest Management in Boreal Fennoscandia: A Review. *AMBIO* 41, 720–737. <https://doi.org/10.1007/s13280-012-0289-y>
- Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karlton, E., Lindahl, B.D., 2017. Shift in fungal communities and associated enzyme activities along an age gradient of managed *Pinus sylvestris* stands. *ISME J* 11, 863–874. <https://doi.org/10.1038/ismej.2016.184>
- Kårén, O., Nylund, J.-E., 1997. Effects of ammonium sulphate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in southwestern Sweden. *Can. J. Bot.* 75, 1628–1642. <https://doi.org/10.1139/b97-875>
- Kõljalg, U., Dahlberg, A., Taylor, A.F.S., Larsson, E., Hallenberg, N., Stenlid, J., Larsson, K.-H., Fransson, P.M., Kårén, O., Jonsson, L., 2000. Diversity and abundance of resupinate theleporoid fungi as ectomycorrhizal symbionts in Swedish boreal forests. *Molecular Ecology* 9, 1985–1996. <https://doi.org/10.1046/j.1365-294X.2000.01105.x>
- Larsson, A. (red), Bjelke, U., Dahlberg, A., Sandström, J., 2011. Tillståndet i skogen – rödlistade arter i ett nordiskt perspektiv. ArtDatabanken Rapporterar 9. ArtDatabanken SLU, Uppsala

- Li, D.-W., 2005. Release and dispersal of basidiospores from *Amanita muscaria* var. *alba* and their infiltration into a residence. *Mycological Research* 109, 1235–1242. <https://doi.org/10.1017/S0953756205003953>
- Lindahl, B.D., Kyaschenko, J., Varenus, K., Clemmensen, K.E., Dahlberg, A., Karlton, E., Stendahl, J., 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. *Ecology Letters* 24, 1341–1351. <https://doi.org/10.1111/ele.13746>
- Luginbuehl, L.H., Oldroyd, G.E.D., 2017. Understanding the Arbuscule at the Heart of Endomycorrhizal Symbioses in Plants. *Current Biology* 27, R952–R963. <https://doi.org/10.1016/j.cub.2017.06.042>
- Luoma, D.L., Stockdale, C.A., Molina, R., Eberhart, J.L., 2006. The spatial influence of *Pseudotsuga menziesii* retention trees on ectomycorrhiza diversity. *Can. J. For. Res.* 36, 2561–2573. <https://doi.org/10.1139/x06-143>
- Mason, W.L., Diaci, J., Carvalho, J. and Valkonen, S. 2022. Continuous cover forestry in Europe: usage and the knowledge gaps and challenges to wider adoption. *Forestry: An International Journal of Forest Research*, 95 (1), 1-12.
- Macherey Nagel™. (2017). NucleoSpin™ Soil DNA Extraction Kit [DNA extraction kit for soil samples].
- Macherey Nagel™. 2017. NucleoSpin™ Soil: Genomic DNA from soil. User manual. Rev. 07
- Mueller, G.M., Cunha, K.M., May, T.W., Allen, J.L., Westrip, J.R.S., Canteiro, C., Costa-Rezende, D.H., Drechsler-Santos, E.R., Vasco-Palacios, A.M., Ainsworth, A.M., Alves-Silva, G., Bungartz, F., Chandler, A., Gonçalves, S.C., Krisai-Greilhuber, I., Iršénaitė, R., Jordal, J.B., Kosmann, T., Lendemer, J., McMullin, R.T., Mešić, A., Motato-Vásquez, V., Ohmura, Y., Næsborg, R.R., Perini, C., Saar, I., Simijaca, D., Yahr, R., Dahlberg, A., 2022. What Do the First 597 Global Fungal Red List Assessments Tell Us about the Threat Status of Fungi? *Diversity* 14, 736. <https://doi.org/10.3390/d14090736>
- Niklasson, M., Granström, A. 2000. ‘Numbers and sizes of fires: Long-term spatially explicit fire history in a swedish boreal landscape’, *Ecology*, 81, 1484–1499. [https://doi.org/10.1890/0012-9658\(2000\)081\[1484:NASOFL\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[1484:NASOFL]2.0.CO;2)
- Nilsson, L.O., Wallander, H., 2003. Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist* 158, 409–416. <https://doi.org/10.1046/j.1469-8137.2003.00728.x>

- Nilsson, L.O., Giesler, R., Baath, E., Wallander, H., 2005. Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. *New Phytol.* 165, 613–622. <https://doi.org/10.1111/j.1469-8137.2004.01233.x>
- Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., & Weedon, J., 2022. *vegan: Community Ecology Package* (Version 2.6-4). <https://CRAN.R-project.org/package=vegan>.
- Peter, M., Ayer, F., Egli, S., 2001. Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. *New Phytologist* 149, 311–325. <https://doi.org/10.1046/j.1469-8137.2001.00030.x>
- Peay, K.G., Kennedy, P.G., Bruns, T.D., 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecol.* 4, 233–240. <https://doi.org/10.1016/j.funeco.2010.09.010>
- Peay, K.G., Schubert, M.G., Nguyen, N.H., Bruns, T.D., 2012. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Molecular Ecology* 21, 4122–4136. <https://doi.org/10.1111/j.1365-294X.2012.05666.x>
- Perotto, S., Daghino, S., Martino, E., 2018. Ericoid mycorrhizal fungi and their genomes: another side to the mycorrhizal symbiosis? *New Phytologist* 220, 1141–1147. <https://doi.org/10.1111/nph.15218>
- Pickles, B.J., Genney, D.R., Potts, J.M., Lennon, J.J., Anderson, I.C., Alexander, I.J., 2010. Spatial and temporal ecology of Scots pine ectomycorrhizas. *New Phytologist* 186, 755–768. <https://doi.org/10.1111/j.1469-8137.2010.03204.x>
- Pinheiro, J., Bates, D., R Core Team. 2022. *nlme: Linear and Nonlinear Mixed Effects Models* (Version 3.1-160). Retrieved from <https://CRAN.R-project.org/package=nlme>
- R Core Team., 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from <https://www.R-project.org/>
- Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist* 157, 475–492. <https://doi.org/10.1046/j.1469-8137.2003.00704.x>

- Rinaldi, A.C., Comadini, O., Kuyper, T.W., 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. *Fungal Diversity* 33, 1–45.
- Saari, S.K., Campbell, C.D., Russell, J., Alexander, I.J., Anderson, I.C., 2005. Pine microsatellite markers allow roots and ectomycorrhizas to be linked to individual trees. *New Phytologist* 165, 295–304. <https://doi.org/10.1111/j.1469-8137.2004.01213.x>
- Santalahti, M., Sun, H., Jumpponen, A., Pennanen, T., Heinonsalo, J., 2016. Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil. *FEMS Microbiology Ecology* 92, fiw170. <https://doi.org/10.1093/femsec/fiw170>
- Sayers, E. W., Bolton, E. E., Brister, J. R., Canese, K., Chan, J., Comeau, D. C., Connor, R., Funk, K., Kelly, C., Kim, S., Madej, T., Marchler-Bauer, A., Lanczycki, C., Lathrop, S., Lu, Z., Thibaud-Nissen, F., Murphy, T., Phan, L., Skripchenko, Y., Tse, T., Wang, J., Williams, R., Trzwick, B. W., Pruitt, K. D., Sherry, S. T. 2022. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 50, D20-D26. <https://doi:10.1093/nar/gkab1112>.
- SLU Artdatabanken, 2020. Rödlstade arter i Sverige 2020. SLU, Uppsala
- SLU Artdatabanken, 2022. URL <https://www.artdatabanken.se/arter-och-natur/Dagens-natur/manga-skogslevande-arter-hotas-av-trakthyggesbruk/> (accessed 2.21.23).
- SLU Artdatabanken, 2023a. Filtrera arter - Naturvård från SLU Artdatabanken. URL <https://artfakta.se/naturvard/filter?organismGroups=%5B7023%5D&redlistCategories=%5B2,3,4,5%5D&landscapeTypes=%5B662%5D&landscapeImportant=true&ecologicGroups=%5B1865%5D> (accessed 2.21.23).
- SLU Artdatabanken, 2023b. Filtrera arter - Naturvård från SLU Artdatabanken. URL <https://artfakta.se/naturvard/filter?organismGroups=%5B7023%5D&ecologicGroups=%5B1865%5D> (accessed 2.21.23).
- Skogsstyrelsen, 2023-04-13. Hyggesfritt skogsbruk. <https://www.skogsstyrelsen.se/mer-om-skog/hyggesfritt/> (accessed 4.24.23).
- Smith, G.R., Steidinger, B.S., Bruns, T.D., Peay, K.G., 2018. Competition–colonization tradeoffs structure fungal diversity. *ISME J* 12, 1758–1767. <https://doi.org/10.1038/s41396-018-0086-0>
- Soudzilovskaia NA, Vaessen S, van't Zelfde M, Raes N. 2017. Global patterns of mycorrhizal distribution and their environmental drivers. In: Tedersoo L, ed. *Biogeography of mycorrhizal symbiosis*. Ecological studies. Cham, Switzerland: Springer International, 223–235.

- Sterkenburg, E., Clemmensen, K.E., Lindahl, B.D., Dahlberg, A., 2019. The significance of retention trees for survival of ectomycorrhizal fungi in clear-cut Scots pine forests. *Journal of Applied Ecology* 56, 1367–1378. <https://doi.org/10.1111/1365-2664.13363>
- Straatsma, G., Krisai-Greilhuber, I., 2003. Assemblage structure, species richness, abundance, and distribution of fungal fruit bodies in a seven year plot-based survey near Vienna. *Mycological Research* 107, 632–640. <https://doi.org/10.1017/S0953756203007767>
- Tedersoo, L., Smith, E. M., 2013. Lineages of ectomycorrhizal fungirevisited: Foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biol. Rev.* 27, 83–99.
- van Schöll, L., Kuyper, T.W., Smits, M.M., Landeweert, R., Hoffland, E., Breemen, N. van, 2008. Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant Soil* 303, 35–47. <https://doi.org/10.1007/s11104-007-9513-0>
- Varenus, K., Lindahl, B.D., Dahlberg, A., 2017. Retention of seed trees fails to lifeboat ectomycorrhizal fungal diversity in harvested Scots pine forests. *FEMS Microbiology Ecology*. <https://doi.org/10.1093/femsec/fix105>
- Vašutová, M., Mleczko, P., López-García, A., Maček, I., Boros, G., Ševčík, J., Fujii, S., Hackenberger, D., Tuf, I.H., Hornung, E., Páll-Gergely, B., Kjøller, R., 2019. Taxi drivers: the role of animals in transporting mycorrhizal fungi. *Mycorrhiza* 29, 413–434. <https://doi.org/10.1007/s00572-019-00906-1>
- Wallander, H., Johansson, U., Sterkenburg, E., Brandström Durling, M., Lindahl, B.D., 2010. Production of ectomycorrhizal mycelium peaks during canopy closure in Norway spruce forests. *New Phytologist* 187, 1124–1134. <https://doi.org/10.1111/j.1469-8137.2010.03324.x>
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Henry, L., 2019. Welcome to the tidyverse. *Journal of Open Source Software*, 4(43), 1686. doi: 10.21105/joss.01686
- Östlund, L., Zackrisson, O., Axelsson, A.-L., 1997. The history and transformation of a Scandinavian boreal forest landscape since the 19th century. *Can. J. For. Res.* 27, 1198–1206. <https://doi.org/10.1139/x97-070>

Invisible underground diversity on clearcuttings?

Have you heard of the underground collaboration going on between trees and certain fungi called mycorrhiza? Then you might wonder what happens to the mycorrhizal community when a forest is clearcut, and by that taking away the food source for the mycorrhiza species? And you might wonder if any mycorrhizal species can survive on the single retention trees that usually are left on clearcuttings? Apart from the visible fruiting bodies, mycorrhizal fungi consist of soil dwelling thin threads, called hyphae, which grows around the thin root tips of their host plant, and form a link, through which they can pass nutrients and minerals to each other. There are several types of mycorrhizae, ectomycorrhiza is a common type to find on tree roots in the northern hemisphere. It is known that the number of ectomycorrhiza species decline, and the community composition is altered by clearcutting. And there are observations of a higher number of species close to single trees, than further away, and that the mycorrhizal community within 1 m of the tree resembles that of an old growth forest. As I am curious, I decided to look at this on a finer spatial scale than has been done before. I achieved this by visiting 4 Scots pine forests in Dalarna country in Sweden that partly had been felled 1-2 years ago. On the clearcuttings I collected soil samples at 1, 3, 7, 15, and 30 m distance from the old growth Scots pine forest and at the same distances around single pine trees. I then extracted DNA from the soil, and from that identified which species that were present at different distances. I observed that the number of ectomycorrhiza species decline with increasing distance from forest edge and with increasing distance from the retention trees. Some species appear to live on the clearcutting, which I believe is a result of some small seedlings that remained from before the felling. The mycorrhizal community on the clearcutting differs from that in the old growth forest. While the single trees share most of their species with the clearcut. *Cortinarius* and *Russula* are the two genera which decline the most in species numbers along these gradients. The conclusion is that clearcutting affects fungal communities associated with old-growth forest and that single retention trees can lifeboat a few more species compared to a clearcut without retention trees, but they have a small effect in preserving fungal communities associated with old growth forest. As I saw a change in community composition between 3-7 meters from the forest edge, but not at closer distances, it would be possible to remove a few trees from an old growth forest without affecting the community in total. However by doing so, as most of ectomycorrhizal species are rare, some species

are likely to disappear by chance. Next thing I would be curious to know, is if or how this changes over time, and also if the same results could be obtained for sites dominated by other tree species as well.

Acknowledgements

First, I would like to send thanks to Anders Dahlberg for sharing your knowledge in such a kindly manner. Great thanks to Line Djupström for the good company and teamwork during the fieldwork. I would also like to send thanks to Yasaman Najafi for your good humour and for taking care of me during my time in the lab. And lastly, I would like to send special thanks to Jesper Wadstein for all your support and for continuously giving me constructive feedback and thoughts on my work.

Appendix 1

Table 5. The table shows the top 20 most frequent ECM species for all samples. In total there are 195 samples. The column with the title Nr. samples, shows the total number of samples of all 195 samples, in which a species occurs. Frequency equals the percentage of the total number of samples a species occurs in.

Species name	Redlist	Nr. samples	Frequency
<i>Cenococcum geophilum</i> (coll.)	LC	112	58,0
<i>Piloderma sphaerosporum</i>	LC	94	49,0
<i>Russula decolorans</i>	LC	82	42,7
<i>Suillus variegatus</i>	LC	69	35,9
<i>Tylospora fibrillosa</i>	LC	57	29,7
<i>Tylospora</i> sp.	LC	50	26,0
<i>Cortinarius caperatus</i>	LC	44	22,9
<i>Russula paludosa</i>	LC	42	21,9
<i>Cortinarius</i> aff. <i>acutus</i>	LC	41	21,0
<i>Piloderma olivaceum</i>	LC	32	17,0
<i>Cortinarius semisanguineus</i>	LC	26	13,5
<i>Cortinarius</i> aff. <i>obtusus</i>	LC	22	11,5
<i>Piloderma bicolor</i>	LC	18	9,4
<i>Tomentellopsis submollis</i>	LC	16	8,0
<i>Russula vinosa</i>	LC	14	7,3
<i>Lactarius rufus</i>	LC	14	7,3
<i>Hyaloscypha finlandica</i>	LC	14	7,3
<i>Ceratobasidium</i>	LC	14	7,3
<i>Cortinarius</i> aff. <i>mucifluus</i>	LC	14	7,0
<i>Hebeloma velutipes</i> (coll.)	LC	13	6,8
<i>Russula emetica</i> (coll.) -			
<i>atrorubens</i>	LC	11	5,7
<i>Byssoporia</i> aff. <i>terrestris</i>	LC	11	5,7
<i>Tylospora asterophora</i>	LC	10	5,2
<i>Elaphomyces asperulus</i>	LC	10	5,2
<i>Lactarius camphoratus</i>	LC	9	4,7
<i>Amanita porphyria</i>	LC	9	4,7
<i>Cortinarius</i> sp.1		8	4,2
<i>Cortinarius biformis</i>	LC	8	4,2
<i>Thaxterogaster pinophilus</i>	LC	7	3,6
<i>Piloderma byssinum</i>	LC	7	3,6
<i>Lactarius vietus</i>	LC	7	3,6

<i>Cortinarius croceus</i>	LC	7	3,6
<i>Tomentellopsis echinospora</i>	LC	6	3,1
<i>Tomentella sp.1</i>		6	3,1
<i>Inocybe subcarpta</i>	LC	6	3,1
<i>Cortinarius testaceofolius</i>	LC	6	3,1
<i>Thelephora terrestris</i>	LC	5	2,6
<i>Suillus flavidus</i>	LC	5	2,6
<i>Rhizopogon evadens</i>	NE	5	2,6
<i>Hygrophorus camarophyllus</i>	LC	5	2,6
<i>Cortinarius coleoptera</i>	LC	5	2,6
<i>Cortinarius clarobrunneus</i>	LC	5	2,6
<i>Chroogomphus aff. rutilus</i>	NE	5	2,6
<i>Amphinema byssoides</i>	LC	5	2,6
<i>Tricholoma aestuans</i>	LC	4	2,1
<i>Tomentella terrestris</i>	NA	4	2,1
<i>Tomentella sp.2</i>		4	2,1
<i>Pseudotomentella humicola</i>	NE	4	2,1
<i>Phellodon niger</i>	VU	4	2,1
<i>Hyaloscypha sp.</i>		4	2,1
<i>Cortinarius traganus</i>	LC	4	2,1
<i>Cortinarius quarcticus</i>	LC	4	2,1
<i>Cortinarius mucosus/alpinus/fennoscandicus/trivialis</i>		4	2,1
<i>Amphinema sp.</i>		4	2,1
<i>Amanita virosa</i>	LC	4	2,1
<i>Tomentella lapida</i>	NA	3	2,0
<i>Tricholoma sp.</i>		3	1,6
<i>Russula taigarum</i>	LC	3	1,6
<i>Russula griseascens</i>	LC	3	1,6
<i>Russula aquosa</i>	LC	3	1,6
<i>Ramaria sp.</i>		3	1,6
<i>Hydnellum ferrugineum</i>	LC	3	1,6
<i>Cortinarius sp.3</i>		3	1,6
<i>Cortinarius sp.2</i>		3	1,6
<i>Cortinarius malachius</i>	LC	3	1,6
<i>Cortinarius causticus</i>	LC	3	1,6
<i>Cortinarius armillatus</i>	LC	3	1,6
<i>Clavulina sp.1</i>		3	1,6
<i>Trichophaea sp.1</i>		2	1,0
<i>Tricholoma stans</i>	LC	2	1,0

<i>Suillus bovinus</i>	LC	2	1,0
<i>Russula sp.</i>		2	1,0
<i>Russula rhodopus</i>	LC	2	1,0
<i>Russula puellaris</i>	LC	2	1,0
<i>Russula consobrina</i>	LC	2	1,0
<i>Russula</i>			
<i>clavipes/nuoljae/pascua</i>		2	1,0
<i>Russula aquosa</i>	LC	2	1,0
<i>Piloderma sp.1</i>		2	1,0
<i>Phellodon sp.</i>		2	1,0
<i>Lactarius helvus</i>	LC	2	1,0
<i>Laccaria laccata (coll.)</i>	LC	2	1,0
<i>Inocybe sp.1</i>		2	1,0
<i>Elaphomyces muricatus</i>	LC	2	1,0
<i>Elaphomyces leveillei</i>	NT	2	1,0
<i>Cortinarius sp.8</i>		2	1,0
<i>Cortinarius sp.7</i>		2	1,0
<i>Cortinarius sp.6</i>		2	1,0
<i>Cortinarius sp.5</i>		2	1,0
<i>Cortinarius sp.4</i>		2	1,0
<i>Cortinarius iliopodius</i>	NA	2	1,0
<i>Cortinarius gentilis</i>	LC	2	1,0
<i>Cortinarius flexipes</i>	LC	2	1,0
<i>Cortinarius brunneifolius</i>	NE	2	1,0
<i>Clavulina sp.2</i>		2	1,0
<i>Chroogomphus rutilus</i>	NE	2	1,0
<i>Trichophaea sp.2</i>		1	0,5
<i>Tomentellopsis zygodesmoides</i>	LC	1	0,5
<i>Tomentellopsis sp.</i>		1	0,5
<i>Tomentella sp.</i>		1	0,5
<i>Sistotrema sp.</i>		1	0,5
<i>Russula versicolor</i>	LC	1	0,5
<i>Russula claroflava</i>	LC	1	0,5
<i>Piloderma sp.3</i>		1	0,5
<i>Piloderma sp.2</i>		1	0,5
<i>Phlegmacium sp.</i>		1	0,5
<i>Phellodon melaleucus</i>	LC	1	0,5
<i>Otidea leporina</i>	LC	1	0,5
<i>Otidea cantharella</i>	LC	1	0,5
<i>Lactarius trivialis</i>	LC	1	0,5
<i>Lactarius torminosus</i>	LC	1	0,5

<i>Inocybe sp.2</i>		1	0,5
<i>Hygrophorus pustulatus</i>	LC	1	0,5
<i>Hydnellum peckii</i>	LC	1	0,5
<i>Hydnellum gracilipes</i>	VU	1	0,5
<i>Hydnellum caeruleum</i>	NT	1	0,5
<i>Hydnellum aurantiacum</i>	NT	1	0,5
<i>Cortinarius suberi</i>	LC	1	0,5
<i>Cortinarius sp.9</i>		1	0,5
<i>Cortinarius sp.20</i>		1	0,5
<i>Cortinarius sp.19</i>		1	0,5
<i>Cortinarius sp.18</i>		1	0,5
<i>Cortinarius sp.17</i>		1	0,5
<i>Cortinarius sp.16</i>		1	0,5
<i>Cortinarius sp.15</i>		1	0,5
<i>Cortinarius sp.14</i>		1	0,5
<i>Cortinarius sp.13</i>		1	0,5
<i>Cortinarius sp.12</i>		1	0,5
<i>Cortinarius sp.11</i>		1	0,5
<i>Cortinarius sp.10</i>		1	0,5
<i>Cortinarius sp.</i>		1	0,5
<i>Cortinarius praestigiosus</i>	LC	1	0,5
<i>Cortinarius neofurvolaeus</i>	LC	1	0,5
<i>Cortinarius luteo-ornatus</i>	LC	1	0,5
<i>Cortinarius limonius</i>	LC	1	0,5
<i>Cortinarius fulvescens (coll.) - tenuifulvescens</i>	LC	1	0,5
<i>Cortinarius angelesianus</i>	LC	1	0,5
<i>Cortinarius abiegns</i>		1	0,5

Table 6. The table shows the top 20 most frequent ECM species for samples taken along forest edges. There are 120 samples in total, 30 at each site, 20 samples for each distance category. The table lists the numbers of occurrences at different distances from forest edge. The column with the title "total" shows the sum of occurrences for each species. The total number of ECM species for each distance category are summed together in the bottom of the table.

Species name	Redlist	-30	1	3	7	15	30	Total
<i>Cenococcum geophilum (coll)</i>	LC	15	13	15	11	8	11	73
<i>Piloderma sphaerosporum</i>	LC	16	16	15	4	3	3	62
<i>Suillus variegatus</i>	LC	17	14	12	4	2	2	50
<i>Russula decolorans</i>	LC	4	8	9	9	10	10	45
<i>Tylospora sp.</i>	LC	7	9	10	4	2	2	35
<i>Tylospora fibrillose</i>	LC	5	8	8	3	3	3	31

<i>Cortinarius aff. acutus</i>	LC	11	7	7	2	1	1	28
<i>Cortinarius caperatus</i>	LC	5	7	7	2	3	3	27
<i>Piloderma olivaceum</i>	LC	11	6	4	0	1	1	24
<i>Russula paludosa</i>	LC	2	2	3	5	4	4	21
<i>Cortinarius semisanguineus</i>	LC	4	6	5	1	4	4	20
<i>Cortinarius aff. obtusus</i>	LC	3	5	4	2	2	2	17
<i>Piloderma bicolor</i>	LC	6	3	1	1	0	0	12
<i>Tomentellopsis submollis</i>	LC	1	2	6	1	0	0	11
<i>Lactarius rufus</i>	LC	2	3	4	1	1	1	11
<i>Hyaloscypha finlandica</i>	LC	2	1	1	3	2	2	11
<i>Hebeloma velutipes (coll.)</i>	LC	3	6	1	0	0	0	10
<i>Ceratobasidium</i>	LC	1	1	3	2	0	0	9
<i>Cortinarius aff. mucifluus</i>	LC	4	0	2	0	2	2	8
<i>Tylospora asterophora</i>	LC	1	4	1	0	2	2	8
<i>Russula vinosa</i>	LC	1	1	2	1	1	1	7
<i>Russula emetica (coll.)</i>	-							
<i>atrорubens</i>	LC	1	0	3	2	0	0	7
<i>Amanita porphyria</i>	LC	3	3	0	0	0	0	7
<i>Thaxterogaster pinophilus</i>	LC	4	2	1	0	0	0	7
<i>Piloderma byssinum</i>	LC	3	2	2	0	0	0	7
<i>Lactarius vietus</i>	LC	3	1	0	1	0	0	7
<i>Byssoporia aff. terrestris</i>	LC	3	2	1	0	0	0	6
<i>Cortinarius croceus</i>	LC	3	0	2	0	1	1	6
<i>Tomentellopsis echinospora</i>	LC	0	1	1	3	0	0	6
<i>Lactarius camphoratus</i>	LC	0	0	2	1	1	1	5
<i>Cortinarius biformis</i>	LC	1	3	1	0	0	0	5
<i>Cortinarius testaceofolius</i>	LC	2	2	0	0	1	1	5
<i>Suillus flavidus</i>	LC	2	1	2	0	0	0	5
<i>Cortinarius clarobrunneus</i>	LC	2	1	1	1	0	0	5
<i>Elaphomyces asperulus</i>	LC	1	1	0	1	1	1	4
<i>Cortinarius sp.1</i>		1	1	1	0	0	0	4
<i>Inocybe subcarpta</i>	LC	0	1	2	0	0	0	4
<i>Hygrophorus camarophyllus</i>	LC	0	1	2	1	0	0	4
<i>Cortinarius coleoptera</i>	LC	0	2	1	0	0	0	4
<i>Chroogomphus aff. rutilus</i>	NE	1	0	2	1	0	0	4
<i>Amphinema byssoides</i>	LC	1	1	1	1	0	0	4
<i>Phellodon niger</i>	VU	0	3	0	1	0	0	4
<i>Cortinarius traganus</i>	LC	0	2	0	1	1	1	4
<i>Cortinarius quarcticus</i>	LC	4	0	0	0	0	0	4

<i>Cortinarius</i>								
<i>mucosus/alpinus/fennoscandicu</i>								
<i>s/trivialis</i>		3	0	1	0	0	0	4
<i>Tricholoma aestuans</i>	LC	2	1	0	0	0	0	3
<i>Tomentella terrestris</i>	NA	0	1	1	0	1	1	3
<i>Pseudotomentella humicola</i>	NE	1	0	0	1	0	0	3
<i>Hyaloscypha sp.</i>		0	1	1	0	1	1	3
<i>Tomentella lapida</i>	NA	1	1	1	0	0	0	3
<i>Russula taigarum</i>	LC	0	1	1	0	1	1	3
<i>Russula griseascens</i>	LC	1	0	1	1	0	0	3
<i>Hydnellum ferrugineum</i>	LC	0	1	0	1	0	0	3
<i>Cortinarius malachus</i>	LC	1	2	0	0	0	0	3
<i>Cortinarius causticus</i>	LC	2	0	0	0	0	0	3
<i>Tomentella sp.1</i>		0	1	1	0	0	0	2
<i>Rhizopogon evadens</i>	NE	0	0	0	1	0	0	2
<i>Russula aquosa</i>	LC	1	0	1	0	0	0	2
<i>Ramaria sp.</i>		0	1	1	0	0	0	2
<i>Cortinarius sp.3</i>		0	1	1	0	0	0	2
<i>Clavulina sp.1</i>		1	0	0	0	1	1	2
<i>Trichophaea sp.1</i>		0	1	1	0	0	0	2
<i>Tricholoma stans</i>	LC	0	1	0	0	0	0	2
<i>Russula rhodopus</i>	LC	0	1	1	0	0	0	2
<i>Russula consobrina</i>	LC	1	0	1	0	0	0	2
<i>Russula aquosa</i>	LC	1	1	0	0	0	0	2
<i>Phellodon sp.</i>		0	0	0	1	0	0	2
<i>Lactarius helvus</i>	LC	0	1	1	0	0	0	2
<i>Laccaria laccata (coll.)</i>	LC	2	0	0	0	0	0	2
<i>Inocybe sp.1</i>		0	1	1	0	0	0	2
<i>Elaphomyces muricatus</i>	LC	0	1	1	0	0	0	2
<i>Elaphomyces leveillei</i>	NT	2	0	0	0	0	0	2
<i>Cortinarius sp.6</i>		1	0	0	1	0	0	2
<i>Cortinarius brunneifolius</i>	NE	0	0	1	0	0	0	2
<i>Clavulina sp.2</i>		1	0	1	0	0	0	2
<i>Thelephora terrestris</i>	LC	0	0	1	0	0	0	1
<i>Tomentella sp.2</i>		0	0	1	0	0	0	1
<i>Amphinema sp.</i>		0	0	1	0	0	0	1
<i>Amanita virosa</i>	LC	0	0	0	0	0	0	1
<i>Cortinarius sp.2</i>		0	1	0	0	0	0	1
<i>Cortinarius armillatus</i>	LC	1	0	0	0	0	0	1
<i>Russula</i>								
<i>clavipes/nuoljae/Pascua</i>		1	0	0	0	0	0	1

<i>Piloderma sp.1</i>		1	0	0	0	0	0	1
<i>Cortinarius sp.8</i>		1	0	0	0	0	0	1
<i>Cortinarius sp.7</i>		0	0	1	0	0	0	1
<i>Cortinarius sp.5</i>		0	1	0	0	0	0	1
<i>Cortinarius iliopodius</i>	NA	0	0	1	0	0	0	1
<i>Cortinarius gentilis</i>	LC	0	0	0	1	0	0	1
<i>Cortinarius flexipes</i>	LC	0	0	1	0	0	0	1
<i>Chroogomphus rutilus</i>	NE	0	0	0	1	0	0	1
<i>Trichophaea sp.2</i>		0	0	0	0	1	1	1
<i>Tomentellopsis zygodesmoides</i>	LC	1	0	0	0	0	0	1
<i>Tomentella sp.</i>		0	0	0	0	0	0	1
<i>Sistotrema sp.</i>		0	1	0	0	0	0	1
<i>Russula versicolor</i>	LC	0	0	0	0	0	0	1
<i>Russula claroflava</i>	LC	1	0	0	0	0	0	1
<i>Piloderma sp.3</i>		0	0	1	0	0	0	1
<i>Piloderma sp.2</i>		0	1	0	0	0	0	1
<i>Phlegmacium sp.</i>		0	1	0	0	0	0	1
<i>Phellodon melaleucus</i>	LC	0	1	0	0	0	0	1
<i>Otidea leporine</i>	LC	0	1	0	0	0	0	1
<i>Otidea cantharella</i>	LC	1	0	0	0	0	0	1
<i>Lactarius trivialis</i>	LC	1	0	0	0	0	0	1
<i>Lactarius torminosus</i>	LC	0	1	0	0	0	0	1
<i>Inocybe sp.2</i>		1	0	0	0	0	0	1
<i>Hygrophorus pustulatus</i>	LC	0	1	0	0	0	0	1
<i>Hydnellum peckii</i>	LC	0	0	0	1	0	0	1
<i>Hydnellum gracilipes</i>	VU	0	1	0	0	0	0	1
<i>Cortinarius suberi</i>	LC	0	1	0	0	0	0	1
<i>Cortinarius sp.9</i>		0	0	1	0	0	0	1
<i>Cortinarius sp.20</i>		0	0	1	0	0	0	1
<i>Cortinarius sp.19</i>		0	1	0	0	0	0	1
<i>Cortinarius sp.18</i>		0	1	0	0	0	0	1
<i>Cortinarius sp.17</i>		0	1	0	0	0	0	1
<i>Cortinarius sp.16</i>		0	1	0	0	0	0	1
<i>Cortinarius sp.15</i>		0	0	1	0	0	0	1
<i>Cortinarius sp.14</i>		0	1	0	0	0	0	1
<i>Cortinarius sp.13</i>		0	1	0	0	0	0	1
<i>Cortinarius sp.12</i>		1	0	0	0	0	0	1
<i>Cortinarius sp.11</i>		1	0	0	0	0	0	1
<i>Cortinarius sp.10</i>		0	0	0	0	0	0	1
<i>Cortinarius praestigiosus</i>	LC	1	0	0	0	0	0	1
<i>Cortinarius neofurvolaesus</i>	LC	1	0	0	0	0	0	1

<i>Cortinarius luteo-ornatus</i>	LC	0	0	1	0	0	0	1
<i>Cortinarius limonius</i>	LC	1	0	0	0	0	0	1
<i>Cortinarius fulvescens</i> (coll.) - <i>tenuifulvescens</i>	LC	0	0	0	1	0	0	1
<i>Cortinarius angelesianus</i>	LC	1	0	0	0	0	0	1
<i>Cortinarius abiagnus</i>		1	0	0	0	0	0	1
<i>Tricholoma sp.</i>		0	0	0	0	0	0	0
<i>Suillus bovinus</i>	LC	0	0	0	0	0	0	0
<i>Russula sp.</i>		0	0	0	0	0	0	0
<i>Russula puellaris</i>	LC	0	0	0	0	0	0	0
<i>Cortinarius sp.4</i>		0	0	0	0	0	0	0
<i>Tomentellopsis sp.</i>		0	0	0	0	0	0	0
<i>Hydnellum caeruleum</i>	NT	0	0	0	0	0	0	0
<i>Hydnellum aurantiacum</i>	NT	0	0	0	0	0	0	0
<i>Cortinarius sp.</i>		0	0	0	0	0	0	0
Nr. ECM Species		68	72	66	38	27	27	298

Table 1. The table shows the top 20 most frequent ECM species for samples taken around retention trees. There are 120 samples in total, 30 at each site, 20 samples for each distance category. The table lists the numbers of occurrences at different distances from the retention trees. The column with the title "total" shows the sum of occurrences for each species. The total number of ECM species for each distance category are summed together in the bottom of the table.

Species name	Redlist	1	3	7	15	30	Total
<i>Cenococcum geophilum</i> (coll.)	LC	12	7	5	5	10	39
<i>Russula decolorans</i>	LC	10	9	8	7	3	37
<i>Piloderma sphaerosporum</i>	LC	11	10	3	4	4	32
<i>Tylospora fibrillose</i>	LC	6	5	7	2	6	26
<i>Russula paludosa</i>	LC	4	6	3	6	2	21
<i>Suillus variegatus</i>	LC	8	9	1	0	1	19
<i>Cortinarius caperatus</i>	LC	5	5	4	2	1	17
<i>Tylospora sp.</i>	LC	7	2	1	2	3	15
<i>Cortinarius aff. acutus</i>	LC	8	3	0	2	0	13
<i>Piloderma olivaceum</i>	LC	4	2	2	0	0	8
<i>Russula vinosa</i>	LC	2	0	2	0	3	7
<i>Cortinarius semisanguineus</i>	LC	3	1	0	1	1	6
<i>Piloderma bicolor</i>	LC	1	1	2	1	1	6
<i>Elaphomyces asperulus</i>	LC	1	2	0	2	1	6
<i>Cortinarius aff. obtusus</i>	LC	3	2	0	0	0	5
<i>Tomentellopsis submollis</i>	LC	2	2	1	0	0	5
<i>Ceratobasidium</i>	LC	0	1	1	1	2	5
<i>Byssoporia aff. terrestris</i>	LC	1	2	1	0	1	5
<i>Russula emetica</i> (coll.) - <i>atrорubens</i>	LC	2	1	1	0	0	4

<i>Lactarius camphoratus</i>	LC	2	2	0	0	0	4
<i>Cortinarius sp.1</i>		1	2	0	1	0	4
<i>Tomentella sp.1</i>		2	1	0	0	1	4
<i>Thelephora terrestris</i>	LC	0	1	1	0	2	4
<i>Lactarius rufus</i>	LC	0	1	0	1	1	3
<i>Hyaloscypha finlandica</i>	LC	1	1	0	0	1	3
<i>Hebeloma velutipes (coll.)</i>	LC	2	1	0	0	0	3
<i>Cortinarius biformis</i>	LC	0	0	1	0	2	3
<i>Rhizopogon evadens</i>	NE	0	0	1	0	2	3
<i>Tomentella sp.2</i>		1	0	0	1	1	3
<i>Amphinema sp.</i>		0	0	2	0	1	3
<i>Amanita virosa</i>	LC	2	1	0	0	0	3
<i>Tricholoma sp.</i>		0	0	1	0	2	3
<i>Tylospora asterophora</i>	LC	1	0	0	0	1	2
<i>Amanita porphyria</i>	LC	1	1	0	0	0	2
<i>Inocybe subcarpta</i>	LC	1	0	0	1	0	2
<i>Cortinarius sp.2</i>		0	2	0	0	0	2
<i>Cortinarius armillatus</i>	LC	1	0	0	1	0	2
<i>Suillus bovinus</i>	LC	0	1	1	0	0	2
<i>Russula sp.</i>		1	0	1	0	0	2
<i>Russula puellaris</i>	LC	2	0	0	0	0	2
<i>Cortinarius sp.4</i>		2	0	0	0	0	2
<i>Cortinarius aff. mucifluus</i>	LC	0	0	1	0	0	1
<i>Cortinarius croceus</i>	LC	1	0	0	0	0	1
<i>Cortinarius testaceofolius</i>	LC	0	0	0	0	1	1
<i>Hygrophorus camarophyllus</i>	LC	0	0	1	0	0	1
<i>Cortinarius coleoptera</i>	LC	0	0	0	0	1	1
<i>Chroogomphus aff. rutilus</i>	NE	0	1	0	0	0	1
<i>Amphinema byssoides</i>	LC	0	0	1	0	0	1
<i>Tricholoma aestuans</i>	LC	0	1	0	0	0	1
<i>Tomentella terrestris</i>	NA	0	0	1	0	0	1
<i>Pseudotomentella humicola</i>	NE	0	0	0	1	0	1
<i>Hyaloscypha sp.</i>		0	0	1	0	0	1
<i>Russula aquosa</i>	LC	0	0	0	0	1	1
<i>Ramaria sp.</i>		0	0	1	0	0	1
<i>Cortinarius sp.3</i>		0	1	0	0	0	1
<i>Clavulina sp.1</i>		0	0	0	0	1	1
<i>Russula</i>							
<i>clavipes/nuoljæ/Pascua</i>		1	0	0	0	0	1
<i>Piloderma sp.1</i>		1	0	0	0	0	1
<i>Cortinarius sp.8</i>		0	1	0	0	0	1

<i>Cortinarius sp.7</i>		1	0	0	0	0	1
<i>Cortinarius sp.5</i>		0	1	0	0	0	1
<i>Cortinarius iliopodius</i>	NA	1	0	0	0	0	1
<i>Cortinarius gentilis</i>	LC	0	1	0	0	0	1
<i>Cortinarius flexipes</i>	LC	0	0	1	0	0	1
<i>Chroogomphus rutilus</i>	NE	0	1	0	0	0	1
<i>Tomentellopsis sp.</i>		1	0	0	0	0	1
<i>Hydnellum caeruleum</i>	NT	1	0	0	0	0	1
<i>Hydnellum aurantiacum</i>	NT	0	1	0	0	0	1
<i>Cortinarius sp.</i>		0	1	0	0	0	1
<i>Thaxterogaster pinophilus</i>	LC	0	0	0	0	0	0
<i>Piloderma byssinum</i>	LC	0	0	0	0	0	0
<i>Lactarius vietus</i>	LC	0	0	0	0	0	0
<i>Tomentellopsis echinospora</i>	LC	0	0	0	0	0	0
<i>Suillus flavidus</i>	LC	0	0	0	0	0	0
<i>Cortinarius clarobrunneus</i>	LC	0	0	0	0	0	0
<i>Phellodon niger</i>	VU	0	0	0	0	0	0
<i>Cortinarius traganus</i>	LC	0	0	0	0	0	0
<i>Cortinarius quarcticus</i>	LC	0	0	0	0	0	0
<i>Cortinarius mucosus/alpinus/fennoscandicus/trivialis</i>		0	0	0	0	0	0
<i>Tomentella lapida</i>	NA	0	0	0	0	0	0
<i>Russula taigarum</i>	LC	0	0	0	0	0	0
<i>Russula griseascens</i>	LC	0	0	0	0	0	0
<i>Hydnellum ferrugineum</i>	LC	0	0	0	0	0	0
<i>Cortinarius malachus</i>	LC	0	0	0	0	0	0
<i>Cortinarius causticus</i>	LC	0	0	0	0	0	0
<i>Trichophaea sp.1</i>		0	0	0	0	0	0
<i>Tricholoma stans</i>	LC	0	0	0	0	0	0
<i>Russula rhodopus</i>	LC	0	0	0	0	0	0
<i>Russula consobrina</i>	LC	0	0	0	0	0	0
<i>Russula aquosa</i>	LC	0	0	0	0	0	0
<i>Phellodon sp.</i>		0	0	0	0	0	0
<i>Lactarius helvus</i>	LC	0	0	0	0	0	0
<i>Laccaria laccata (coll.)</i>	LC	0	0	0	0	0	0
<i>Inocybe sp.1</i>		0	0	0	0	0	0
<i>Elaphomyces muricatus</i>	LC	0	0	0	0	0	0
<i>Elaphomyces leveillei</i>	NT	0	0	0	0	0	0
<i>Cortinarius sp.6</i>		0	0	0	0	0	0
<i>Cortinarius brunneifolius</i>	NE	0	0	0	0	0	0

<i>Clavulina sp.2</i>		0	0	0	0	0	0
<i>Trichophaea sp.2</i>		0	0	0	0	0	0
<i>Tomentellopsis zygodesmoides</i>	LC	0	0	0	0	0	0
<i>Tomentella sp.</i>		0	0	0	0	0	0
<i>Sistotrema sp.</i>		0	0	0	0	0	0
<i>Russula versicolor</i>	LC	0	0	0	0	0	0
<i>Russula claroflava</i>	LC	0	0	0	0	0	0
<i>Piloderma sp.3</i>		0	0	0	0	0	0
<i>Piloderma sp.2</i>		0	0	0	0	0	0
<i>Phlegmacium sp.</i>		0	0	0	0	0	0
<i>Phellodon melaleucus</i>	LC	0	0	0	0	0	0
<i>Otidea leporine</i>	LC	0	0	0	0	0	0
<i>Otidea cantharella</i>	LC	0	0	0	0	0	0
<i>Lactarius trivialis</i>	LC	0	0	0	0	0	0
<i>Lactarius torminosus</i>	LC	0	0	0	0	0	0
<i>Inocybe sp.2</i>		0	0	0	0	0	0
<i>Hygrophorus pustulatus</i>	LC	0	0	0	0	0	0
<i>Hydnellum peckii</i>	LC	0	0	0	0	0	0
<i>Hydnellum gracilipes</i>	VU	0	0	0	0	0	0
<i>Cortinarius suberi</i>	LC	0	0	0	0	0	0
<i>Cortinarius sp.9</i>		0	0	0	0	0	0
<i>Cortinarius sp.20</i>		0	0	0	0	0	0
<i>Cortinarius sp.19</i>		0	0	0	0	0	0
<i>Cortinarius sp.18</i>		0	0	0	0	0	0
<i>Cortinarius sp.17</i>		0	0	0	0	0	0
<i>Cortinarius sp.16</i>		0	0	0	0	0	0
<i>Cortinarius sp.15</i>		0	0	0	0	0	0
<i>Cortinarius sp.14</i>		0	0	0	0	0	0
<i>Cortinarius sp.13</i>		0	0	0	0	0	0
<i>Cortinarius sp.12</i>		0	0	0	0	0	0
<i>Cortinarius sp.11</i>		0	0	0	0	0	0
<i>Cortinarius sp.10</i>		0	0	0	0	0	0
<i>Cortinarius praestigiosus</i>	LC	0	0	0	0	0	0
<i>Cortinarius neofurvolaeus</i>	LC	0	0	0	0	0	0
<i>Cortinarius luteo-ornatus</i>	LC	0	0	0	0	0	0
<i>Cortinarius limonius</i>	LC	0	0	0	0	0	0
<i>Cortinarius fulvescens (coll.) - tenuifulvescens</i>	LC	0	0	0	0	0	0
<i>Cortinarius angelesianus</i>	LC	0	0	0	0	0	0
<i>Cortinarius abiegus</i>		0	0	0	0	0	0
Nr ECM Species		39	38	29	18	29	152

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. You will find a link to SLU's publishing agreement here:

- <https://libanswers.slu.se/en/faq/228318>.

YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.