Presence of *Hydnellum* species on pine heaths

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Preface

One of my biggest interests here in life is fungi, not only as a mushroom hunter but first and foremost fungal ecology. After many years of wondering about the difference in fungal distribution in “to the untrained eye” equivalent forests I decided to go back to school to learn more about this. During my education I have learned a lot about fungal ecology and realized that human forest management affects many species which if we continue probably will be driven to extinction. This thesis which is my proof of finishing my biology education making me a biologist, I decided to dedicate to one of this threatened groups of species, the *Hydnellum* spp. My hope is that I in the future will be able to work with protection of environments important for the survival of threatened species, especially the fungal species in old-growth forests, and I hope that my knowledge about fungi, their ecology and the role they play in the relationship with other organisms and their survival will help me in this work.

Abstract

The aim of this study was to assess whether the age, the circumference and the density of trees and stumps in forests affect the occurrence of stipitate hydnoid fungi in the genus *Hydnellum*. This was done by surveying sporocarps of *Hydnellum* species in 19 forest stands of potential different age and forest characteristics on a low productive pine heath in northern Sweden. All other fungal species that was present in the forest stands were also noted. The estimated mean age of the trees in the studied forest stands varied between 25 and 144 years. There was a positive correlation between the number of *Hydnellum* sporocarps, as well as between the number of fungal species observed, and the estimated age and the mean circumference of the trees in the stands. The number of sporocarps of *Hydnellum* species was also positively correlated with the total number of fungal species found. In total, 54 different species of fungi was found and several threatened fungal species other than *Hydnellum* were found in the older stands. The lower number of *Hydnellum* and other fungi in younger stands may be due to these stands being more affected by logging. More thorough studies are needed to evaluate the status and ecological requirements of these fungi to prevent them from further decline.

Keywords: *Hydnellum*, pine heat, forest age, mycorrhizal fungi, stipitate hydnoid fungi, threatened species, red data list.
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1 Introduction

1.1 Background

The forestry practices used in Sweden today with large areas being clear-cut and replanted with monocultures have disadvantages for biodiversity (Sahlin 2011). All around the world there has been a decline in the biodiversity and already more than a decade ago both EU and UN decided that the loss of species should be halted by the year 2010. According to the action plan report from the European Commission (2013) this has not been accomplished and species continue to decline. On the Swedish red list arranged after rules and regulation from IUCN (International Union for Conservation of Nature), there were 4128 species listed in the year 2010 edition (Gärdenfors 2010).

Fungi are a large and diverse group of species and the majority are found in woodland habitats. Out of the more than 5000 species of macro fungi known to occur in Sweden, 746 species are placed on the Swedish red list, and 85% of these occur in forests (Dahlberg et al. 2010). Stipitate hydnoid fungi in the genus *Hydnellum* (Thelephorales, Basidiomycota) is one group of fungi that has shown a negative trend in forest habitats probably due to modern forestry. These fungi are woody mycorrhizal soil fungi that produce relatively long-lived sporocarps with a hymenium of teeth on the underside from which they release their spores (Newton et al. 2002, Holden 2008, van der Linde et al. 2008, 2009, Arnolds 2009).

Some *Hydnellum* species are found on sandy pine heaths were they form ectomycorrhiza (for more information on mycorrhiza see appendix C) with Scots pine (*Pinus sylvestris*), which is very important for the trees in this nutrient poor habitat (Holden 2008). During the past decades these fungi have declined over all Europe. Many *Hydnellum* species are placed on the national red list of several European countries (Arnolds 2009) and many of the species are placed on the European red list (Van der Linde et al. 2008). In some parts of Europe sporocarps of some of the *Hydnellum* species that live in symbiosis with Scots pine have not been found since the 70's and they are believed to be extinct in these areas (Van der Linde et al. 2009). According to Arnolds (2009) several *Hydnellum* species and other hydronoid fungi have gone extinct in the Netherlands. One reason for this decline is assumed to be increased deposition of nitrogen and according to Nitare (2006) are forestry believed to affect *Hydnellum* species negatively and some of them seem to completely disappear after clear cutting. (Nitare 2012 a and b).

In Sweden, clear-cutting started in the late 1940-ies early 1950-ies (Sahlin 2011) so forests with trees older than that have most likely never been clear-cut. Forest stands with a longer continuity of trees are expected to have more fungal species present (Wallander et al. 2010) and according to Nitare (2006), Arnolds (2009), Dahlberg and Stenlid (1995) and Visser (1995). *Hydnellum* species are associated with mature stands. In other words, Forest stands with characteristics indicating that it is a mature stand with tree continuity, may sustain more *Hydnellum* spp. sporocarps than forest stands more affected by modern forestry.

To my knowledge there are no scientific studies published so far from Sweden on how *Hydnellum* is affected by the Swedish forestry model, where forests are clear cut, replaced by
monocultures, thinned and clear cut again with a rotation period of about 80 years (Sahlin 2011).

This study was performed in a pine heath forest close to Skellefteå in northern Sweden where different parcels are of different age and have different management history. Although it is not possible to estimate total population sizes of fungi by monitoring fruit body production in just one year, the results from this study will hopefully contribute to increased knowledge about the ecological requirements of these important mycorrhizal fungi. To improve the status of these fungi and to be able to give guidance for sustainable management, there is a need for increased understanding of factors that are necessary for Hydnellum species to survive and grow.

1.2 Aim
The aim of this study was to gain an understanding of factors that may affect presence of Hydnellum species on pine heaths. In particular, I asked whether there is any correlation between forest characteristics such as size, age, density of trees and stump density in forest stands and sporocarp production of Hydnellum species? These characteristics may indicate the management history of the forest with older and larger trees and lower tree and stump density expected in forest stands with a longer continuity of trees than in a stands with a history of more intense forestry with thinning and clear-cutting.

2 Method and material

2.1 Description of Hydnellum species found on pine heats
Stipitate hydnoid species in the Hydnellum genus occurring on sandy pine heaths are H. caeruleum, H. ferrugileum, H. peckii, H. aurandiacum and H. gracilipes. According to Nitare (2006) many stipitate hydnoide fungi are sensitive to clear-cutting and they are more often found in older forests where there has been a continuity of trees. Not all of these species are found on the Swedish red list. Hydnellum ferrugileum and H. peckii have been downgraded from the red list, even though they are sensitive to forest management and are still on the international red list and classified as extinct in some countries. For example, several Hydnellum species and other hydronoid fungi have gone extinct in the Netherlands (Arnold 2009).

Hydnellum caeruleum
Hydnellum caeruleum, blue toothed fungi, form ectomycorrhizae with both pine and spruce. It occurs mainly in older forests with a long continuity of trees. It is especially frequent on nutrient poor and dry sandy pine heaths with older trees and lichen and lingonberries on the ground. It occurs both on calcareous soil and lime poor soil. This species is considered near threatened and the threat is believed to be both habitat loss and nutrient enrichment of forest soil (Nitare 2012 a). One interesting feature of this fungus is that it seems to be able to switch host and form mycorrhiza also with shrubby species. According to Newton et al. (2002), H. caeruleum has been observed on a completely treeless site with the ericaceous shrub Arctostaphylos uva-ursi.
**Hydnellum ferrugineum**

*Hydnellum ferrugineum*, mealy tooth fungus, is according to Nitare (2006) a less threatened species and is quite common in Sweden. It is found in conifer forests, mainly on nutrient-poor dry sites with pine, but it can also be found in spruce forests. It is, however, missing from several other countries (Arnolds 2009).

**Hydnellum peckii**

*Hydnellum peckii*, bleeding tooth fungus, is also quite common in Swedish forests. It prefers sandy soils with poorly developed humus and a minimal vascular plant cover. It is observed to produce sporocarps more often in older pine stands and in soils with low nitrogen content (Nitare 2006). Nitrogen deposition resulting from atmospheric pollution is believed to be a key factor in the observed decline of *H. peckii* populations, particularly in areas of Europe (Holden 2008).

**Hydnellum aurantiacum**

*Hydnellum aurantiacum*, orange toothed fungi, is mostly found in productive old forest, but is also found on sandy pine heaths with nutrient-poor dry ground. This species is in addition to sensitivity to logging, believed to be sensitive to deposition of nitrogen (Nitare 2012 b).

**Hydnellum gracilipes**

*Hydnellum gracilipes* is a strongly endangered species that is associated with older dry pine forests with a history of fire. It forms mycorrhiza with pine and is very difficult to locate because of its small sporocarps that usually are hidden beneath fallen trunks that often are scarred by fire (Nitare 2012 c).

### 2.2 Study area

The area chosen for this study is a low productive pine heath forest, divided into small parcels owned by different private people. It is located a few km from the coast about thirty km south of the town of Skellefteå in the county of Västerbotten, Northern Sweden. Coordinates RT90: 7173160, 1760483 (Fig. 1). In the study area different forest stands have been managed in different ways as commonly observed when there are several forest owners in a restricted area. This is in contrast to company-owned land which usually consists of large areas managed in a similar way. The study area therefore provides the opportunity to study forest stands with different age and management history within a restricted area, where environmental factors are relatively similar. This reduces the risk that environmental variability influences the results of the study. The study area consisted of nutrient poor podzol soil with a sparse vegetation cover with lingonberry (*Vaccinium vitis-idaea*), common heather (*Calluna vulgaris*) and cup lichens (*Cladonia spp*), and some bryophytes and bilberries (*Vaccinium myrtillus*) were found in some patches. The litter and humus layer is at the thickest around 3 cm deep, Pine is the dominant tree species in the area with scattered occurrence of spruce and birch.

### 2.3 Locating study plots

The area was first studied by aerial-photo and then visited for visual investigation to better locate the borders between parcels. Within the area, nineteen forest stands with similar vegetation cover were chosen for the study. A map and a photo over the area was downloaded from SLU maps and up loaded in-to the program arc view. With help of the photo and
coordinates taken during the ocular study of the area a shape file with a map of the chosen forest stands was created (Fig. 1).

Figure 1. Left; Map of Northern Sweden showing the location of the study area (red quadrat). Right; Image of the study area with the green patches showing the 19 parcels studied (red circle show the coordinates: 7173160, 1760483).

2.4 Inventory and estimation of forest characteristics
The characteristics of the forests were inventoried in 5 randomly chosen circular subplots with a diameter of 20 m within each of the 19 forest stands. The locations of the subplots were chosen by letting a random number generator chose points from a derived map with x and y coordinates. The coordinates were marked on the map in Arc view and located in the field using a GPS. In each subplot presence of all tree species were noted, individual trees counted and the circumference at breast height (1.3 m) of each individual tree was measured using measuring tapes. The density of trees was estimated in each subplot by dividing the total number of trees by the area of the subplot. A mean in each forest stand was estimated by pooling the density of all 5 subplots. Tree density was multiplied by 10 to get trees /10m². Similarly, the mean size of trees in the forests stands was estimated by taking the mean circumference of trees in the five subplots.

In forests stands with larger potentially older trees, two trees within each subplot (10 trees per stand) were drilled with an increment borer to estimate the age of the trees. Normally trees are drilled at breast height, however in areas with low productivity the age of the trees can differ by many years at breast height (Berggren 2004), so the drilling was done as close to the ground as possible. Using a stereo microscope with 60 times magnification, the annual rings on each drilled core were counted to estimate the approximately age of the trees.
Changes in size of the annual rings were also used to determine the latest thinning event in the area.

In stands with smaller younger trees, tree age was estimated by counting the number of branch nodes on two trees in each subplot from the ground to the top of the trees. This method is appropriate to use for young conifer trees like pine as they develop one set of branches each year (Rollinson 2012). To get an exact age of the trees, counting branch nodes is not an appropriate method, and in dense forests, trees often lose their lower branches at an early age and the nodes disappear. In this area, however, the stands are sparse and even trees as old as 60 years still have branches almost down to the ground, which makes this method appropriate to use. To get an idea of the management history and approximate time since last cutting in the forest stands, the number of tree stumps was counted, their stage of decomposition were determined and the amount of dead woody debris was visually estimated in the subplots.

In patches with a diameter of 2 m, the type and cover of the ground vegetation was visually estimated. (See appendix A for more detailed information of each plot).

2.5 Inventory of *Hydnellum* sporocarps

In each of the 19 chosen forest stands, a plot of approximately one hectare was marked using a GPS. The plot was totally inventoried for sporocarps of *Hydnellum* species. Because the shapes of the forest parcels were not the same (Fig. 1) the one hectare study plots got different shapes (Fig. 2). All sporocarps within the study plot were counted and determined to species. Sporocarps of all other species of fungi encountered in the study plots were also recorded. All study plots were inventoried within one week to minimize phenological differences in fruit body production between different study plots.

2.6 Data analysis

The data on forest characteristics and the observed number of sporocarps in the forest stands were not normal distributed. Spearman Rank correlations were, therefore, used to test the relationships between the forest characteristics and the number of *Hydnellum* sporocarps and total sporocarps of all species observed in the forest stands. All statistical tests were done in the program NCSS version 9 (Hinze 2012). Because multiple tests were done on the same data set, p-values were adjusted using Bonferroni correction methods (Abdi 2007). To perform a Bonferroni correction, an online calculation program was used (Sisa 2014) – A new shape file of the study plots and a shape file with all sporocarps found were created in Arc Map with the help of coordinates taken with the GPS during the inventory of *Hydnellum* sporocarps (Fig. 2).
3 Results

3.1 The characteristics of the forest stands
The estimated mean age of the trees in the studied forest stands varied between 25 years in stand 19 to the maximum of 144 years observed in stand 12. The oldest tree was found in stand 10 with an age of about 193 years (Table 1). Estimated mean age and average tree circumference in the forest stands was positive correlated (Fig. 3, Table 2), but in consideration to the estimated age of the trees, most trees were thin (Table 1). In contrast a negative correlation was found between forest age and tree and stump density (Table 2). However, the density of trees was relatively low overall with less than 23 trees per 10 m² in the most dense forest stand (stand 17) and only 4 trees per 10 m² in the most sparse forest stands (stand 3) (Table 1). The largest tree was found in plot 3 and that tree had a circumference of 144 cm. On average stand 8 had the widest trees with a mean circumference of 76.4 cm (Table 1).

3.2 The occurrence of *Hydnellum* species sporocarps
Sporocarps of *Hydnellum* species were found in 13 of the 19 inventoried forests stands (Table 1). In total 272 *Hydnellum* sporocarps was located over all forest stands; 166 of these were *H. caerleleum*, 64 *H. peckii* and 42 *H. ferrugineum* (Fig. 4, Table 1). There was a positive correlation between the number of sporocarps of the *Hydnellum* species observed in total and the estimated mean age (Fig. 5, Table 2) and the circumference (Fig. 6, Table 2) of trees.
in the forest stands. The youngest forest with presence of *Hydnellum* sporocarps was area 15 were one fruit body of *H. ferrugineum* was found. The forest stand with the highest number of *hydnellum* sporocarps (77) was stand 7 with an estimated mean age of about 71 years.

**Figure 3.** The relationship between the mean circumference of the trees and the mean age of the forest stands.

**Figure 4.** The figures show from left; *H. caeruleum, H. ferrugineum and H. peckii*. Pictures were taken by the author during the inventory.

**Table 1.** The characteristics of the studied forest stands, and sporocarps observed in the inventory; The forest characteristics estimated were mean age of trees, age of the oldest tree located, estimated density of trees per 10 m², estimated circumference and max circumference (cm) at breast height (1,3m), estimated stump density (number per 10 m²). The number of sporocarps of the different *Hydnellum* species, the total number of *Hydnellum* spp sporocarps and the number of other fungal species are shown in the table. For more details how variables were estimated see material and methods.

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<th>max age</th>
<th>Tree density</th>
<th>Mean circumference</th>
<th>Max circumference</th>
<th>Stump density</th>
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<th><em>H. ferrugineum</em></th>
<th><em>H. peckii</em></th>
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7
3.3 The occurrence of other fungal species

In total, 54 different species of fungi was found in the 19 forest stands (Appendix B). There was a positive correlation between the mean age of the forest stands, the circumference of tree and the number of fungal species observed in total in the stand (Fig. 7, Table 2). There was also a positive correlation between the number of sporocarps of Hydnellum species and the numbers of other fungal species found in the forest stands (Fig. 8, Table 2). Most fungi
that were found in younger forest stands were even found in the older areas, but several fungi were only found in the older forests. These were Bankera fuligineoalba, Boletopsis grisea, Lactarius musteus, Ramaria spp, Sarcodon squamosus, Tricholoma apium, Tricholoma matsutake and Tricholoma roseoacerbum (Appendix B), which all are species that were classified as vulnerable on the Swedish red list year 2010 (Gärdenfors et al. 2010). One species, Lyophyllum decastes/Peinus, was only found in one of the older stands. In 2008 this species was noticed in Sweden by the Japanese researcher, Etsuko Harada and she classified it to be Hon-shimeji (Lyophyllum shimeji), an appreciated mushroom in Japan (Sundberg 2009). This species may be rare or overlooked in Sweden and whether it will end up on the Swedish red list remains to be seen.

Figure 7. The relationship between the total number of fungal species found and medium age of the forest.

Figure 8. The relationship between the number of Hydnellum sporocarps found and the total number of fungal species found.

4 Discussion
The results of this study clearly show that the characteristics of the forest may affect sporocarp production of species in the genus Hydnellum, which form ectomycorrhiza with coniferous trees. As expected the circumference of trees increase with forest age and a
positive correlation was found between the number of *Hydnellum* spp. sporocarps and the age and circumference of trees in the forest stands (Table 2). No *Hydnellum* sporocarps were observed in forests younger than 50 years. The highest number of sporocarps was, however, found in a forest stand with a mean age of 71 years. There were even many older trees in this stand and the oldest tree according to the drilling was approximately 140 years old.

Clear-cutting is believed to have a negative effect on ectomycorrhizal fungi such as *Hydnellum* which has been put forward as an explanation for the observed decrease of these species in forest habitats (Nitare 2006). In consideration to that Sweden started clear-cutting in the late 1940-ies early 1950-ies (Sahlin 2011) forests older than 70 years in the current study have probably never been clear-cut. The older forest stands had lower stump and tree densities than the younger forest stands, which indicate a longer continuity of trees. This may explain why the older forests had more sporocarps than the younger forests in this study. With removal of the tree symbiont the mycelia of *Hydnellum* species may die. However, it is not possible to say whether the younger stands in this study have been clear-cut or not but the higher stump density indicate that the younger stands have been more affected by forestry than the older stands. According to Nitare (2006) and previous studies done in Fennoscandia (Dahlberg and Stenlid 2010), Netherland (Arnolds 2009) and Canada (Visser 1995), also found that *Hydnellum* species were associated with mature forest stands. Some previous studies have also shown that forest management and forest age influence species richness of ectomycorrhizal fungi in general with more species found in older forest stands with longer continuity of trees than in younger forests more affected by modern forestry (Wallander et al. 2010). In my study, I also found more fungal species in total in the older forests. However, in a study by Newton et al. (2002) sporocarps of a variety of stipitate hydnoid species (including some *Hydnellum* species) were on several occasions also found in association with relatively immature forest stands (<30 years old). In another study done by van der Linde et al. (2009), where they inoculated mycelium in seedlings planted out to new native pine forests, the mycelium were found to survive in the ground for several years after inoculation. At this stage it is hard to say whether removal of the tree symbiont trough logging or clear cutting completely kills ectomycorrhizal fungi or decrease sporocarp production. The study by Newton et al. (2002) suggests that *Hydnellum* species might remain in younger plantation for example by forming mycorrhiza with other species than pine. More studies are needed to evaluate how sporocarp production and mycelia growth is affected by modern forestry.

In Sweden several stipitate hydnoid fungi are still quite common, but according to Nitare (2006) increased nitrogen deposition as well as habitat loss may impose threats to these species. Fertilization has been conducted in Swedish forests since the mid-1960-ies. There was a peak in the 1970-ies when about 190 000 ha was fertilized yearly, but due to questioning by environmental groups and governmental bodies, from an environmental point of view, the fertilization dropped to about 20 000 ha per year in the 1990-ies. This did increase again and by the year 2010 a total of approximately 60 000 ha forest were fertilized every year, and about 2 million ha productive forests had been fertilized one or more times at that time (Jacobson and Pettersson 2010). The most probably causes for many *Hydnellum* and other hydnoid fungi going extinct in the Netherlands is the high amount of nitrogen deposition (Arnolds 2009). In consideration to this, there is a need for studies that investigate how *Hydnellum* species are affected by the increased fertilization of the Swedish forests.
Not all *Hydnellum* species expected to be found in this area of Sweden were found in the study area (e.g. *H. aurantiacum* and *H. gracilipes*). There are many possible causes for their absence in the inventory. One possible explanation might be that these species did not produce sporocarps in 2013, the year of the survey. It has been shown that production of sporocarps can be highly seasonal, and many fungal species produce sporocarps sporadically. Many years surveying may, therefore, be required to document the occurrence of all fungi species in a particular area (Newton et al. 2002). It is also possible that sporocarps were missed in the survey. *Hydnellum gracilipes* is a fungus that is very difficult to locate because of its small sporocarps that usually are hidden beneath fallen trunks (Nitare 2012 c). The study area is extremely dry and has probably a history of burning and traces of fire such as carbon residue on stumps were observed during the study. The strongly endangered *H. gracilipes* is associated to older dry pine forests with a history of fire. The study area seems therefore to be a suitable habitat for this species. To find out whether *H. gracilipes* occur in the area a more thorough survey should be done, preferable in a ‘good’ sporocarp production year.

Sporocarps of fungi were overall scarce in 2013 in this region as well as in other areas with resembling conditions. Areas known to have *Hydnellum* species and other threatened fungal species present, showed low frequency of sporocarps this year (personal observations). The study area is also known to people that pick edible mushrooms. There are even mushroom hunters that pick *Hydnellum* species to dye woollen yarn, so there is always a risk that the real distribution of species is overlooked when presence of sporocarps are used as an estimate of species distributions. To get a more complete picture of what factors that influence the occurrence of *Hydnellum* species, several years of studies are needed. An even better way to survey fungi in a particular area is to use molecular methods to study mycelia in the soil. Fungal DNA in the soil could be extracted and run through a PCR using primers adapted to the specific species. To make sure that the mycelium is metabolically active, RNA could be extracted (Van der Linde et al. 2012). Van der Linde et al. (2008, 2009, 2010, 2012) has conducted several studies were they have developed primers and refined the methods for extracting DNA from hydnoid fungi, so these studies can be used as a guideline in future molecular work on *Hydnellum* species and other threatened hydnoid fungi in Swedish forests.

The reason for choosing the study area for this study was that both very young forests and old forests were found within a limited area. At the first appearance the forests seemed to be much younger than they really were. The area was also not that affected by forestry as expected. Given that the tree stands were sparse in general in the area, I expected to find more stumps than I did. However, there are some pits in the area that may be old charcoal pits, so it’s possible that even the tree roots and stumps were removed once upon a time to make charcoal. According to the Swedish national heritage board (2013) one charcoal pit from around the sixteenth century is registered in the area, but there are several unregistered pits that look like they are of newer age (personal observation).

As mentioned in the introduction, many fungal species are threatened in the Swedish forests today (Dahlberg et al. 2010). The aim of this study was to highlight one of these groups, species among the genera *Hydnellum*. Hopefully the results from my study will lead to the requirement for more knowledge about these species to prevent them from decreasing...
toward extinction as observed in the Netherlands (Arnold 2009). In general it is important to evaluate how all types of management in the forests today affect different species and species diversity.

Acknowledgements
First of all I like to thank my supervisor, Ulla Carlsson-Graner for all the help she has been giving me both in planning the work and in writing the report. And I would also like to thank Anders Dahlberg for advises during the work.

References
Nitare, J 2012 b. Artfaktablad: Hydnellum aurandiacum, orange taggsvamp. ArtDatabanken, SLU.


Sisa. 2014. Calculate Bonferroni correction.


Sundberg, H. 2009. *Phylogeny of Lyophyllum, section Difformia, Does hon-shimeji (L. shimeji) occur in Sweden?*. Degree project for Master of Science in Botanical Systematics and Evolution 30 hec. Department of Plant and Environmental Sciences, University of Gothenburg


Appendix A

Plot 1
The mean tree age in this stand is about 35 years old and the oldest tree located, about 46 years old. The tree density is about 12 trees per 10 m². The area has recently been thinned and the wood left, so the ground has abundant with fresh dead wood. The frequency of tree stumps is quite high with more than 5 larger stumps per 10 m², not including the most fresh ones from the most resent thinning, that all are in the size of less than 20 cm in circumference. Tree stumps occur in all sizes and in all stages of decomposition, some of the largest ones had a diameter of more than 50 cm and was still hard so the area has probably been thinned several times in the past before clear-cutting. Annual rings from the oldest trees show an increased growth around 56 and 41 years ago, which may indicate that thinning and cutting of seed trees was done at those times.

Plot 2
At the first appearance the forest seems to be very young, the trees are small and thin, but after a closer investigation it turns out to be older. The tree density in the stand is about 10 trees per 10 m². The majority of the trees are about 80 years old; the oldest drilled tree about 162 years old and the youngest documented by counting branches of trees about 60 years. There is no sign of resent cutting. The frequency of tree stumps is about 3 per 10 m². All tree stumps are quite degraded, but not in the same degrading stage so trees have been cut down on several occasions in the past. Annual rings show an increased growth for about 70, 50 and 35 years ago, indicating that selective felling (or deforestation, cutting of seed trees and thinning) was done at those times.

Plot 3
In this stand the trees are large and one layered, but there are few trees, only about 4 trees per 10 m². Most trees are old, mean age about 110 years old and the oldest tree drilled about 144 years old. Although there are large areas with no trees there are only few places where seedlings are trying to establish. There are no sign of resent discrimination of establishing trees by cutting, only dead seedlings not managing to survive. The annual rings don’t show any pronounced changes in width after cutting, but there are some signs of some growth increase for about 70-75 and 40-45 years ago. Tree stumps were few and strongly degraded, with only about 3 stumps per 10 m². The forest has apparently been thinned in the past, but no new trees have established.

Plot 4
This stand lies on the side of the esker. Here the forest is sparse, about 5 trees per 10 m² and consists of thin trees with a dominant age of 110 years and the oldest drilled tree is 185 years old. As in plot 3, no new trees are establishing. The youngest tree drilled has a circumference at only 41 cm, but is still at least 110 years old. The thinnest trees, even though they are only 20-30 cm in circumference, the branches stops so high that their age is impossible to estimate by counting the branches, but they are definitely old. There are only few tree stumps, about 2.3 per 10 m², and all are strongly degraded. The annual rings don’t either show any clear signs of periods with increased growth. The small changes in width of the tree rings are from different times (between 60-80 years ago and 30-45 years ago) so if they are due to cuttings, the cutting is possible done by cutting single trees at different times.
Plot 5
This younger forest stand is on the side of the esker. At the first appearance the forest seems to be much younger than it is. The forest is sparse and the trees are only 2-5 meters high although they are 30 to 40 years old. All trees are thin so the age was determined by counting branches and no drilling was done. Tree density was about 10 trees per 10 m². There are few tree stumps, only about 3 per 10 m². All tree stumps are still hard suggesting that they originate from seed trees that were left during felling and have been cut down at a later date. No older tree stumps are visible. Dating the latest logging event is difficult without drilling, but looking at the age of the forest and the hardness of the stumps the latest cutting was probably done for a maximum of 20-30 years ago.

Plot 6
The age of most of the trees in this stand is between 30 and 40 years old. The oldest tree located is about 45 years. The forest is sparse with just more than 11 trees larger than two meters per 10 m². But the frequency of seedlings smaller than 2 m is quite high; many are however severely damaged by browsing moose. Tree stumps are quite abundant, about 6 per 10 m². Most stumps are almost totally degraded, but a few fresher. According to the annual rings the oldest trees grew slower from the beginning, and about 25-30 years ago their growth increased, indicating that this area has been left with seed trees when the felling was done who again was felled about 30 years ago.

Plot 7
Most of the trees in this stand are about 70 years old, but the abundance of older trees is quite high. The oldest tree located is at least 140 years old. Tree density is about 11,5 trees per 10 m². Tree stumps are scarce only about 3,2 per 10 m². The annual rings show weak signs of increased growth for about 75, 45 and 30 years ago, indicating selective felling. The ground vegetation is extremely scarce and large areas are completely clean from both ground vegetation and trees. There are no sign of resent discrimination of establishing trees by cutting, so in this area, new plants apparently have difficult to establish.

Plot 8
This is a one layered forest with a mean age of about 145 years and the oldest tree drilled about 178 years old. The trees are scarce only about 5,2 trees per 10 m², and few seedlings are regenerating the area. There are few tree stumps, only about 2 per 10 m² and no sign of thinning. tree rings show few signs of increased growth and the small growth increase shown come at different times so if they are due to cuttings it is probably from selective cutting on various occasions. Latest growth increase was at least 50 years ago.

Plot 9
This forest has recently been thinned, yet most trees in the area are older than 110 years and the oldest tree drilled about 150 years old. The tree density is about 5 trees per 10 m². Fresh dead woody debris is spread throughout the area and the ground is disturbed by tracks were the forwarder have been driving. Tree stumps are frequent with more than 5 stumps per 10 m²; most fresh but also a few that are older from earlier feelings. Before the thinning, this stand was probably one of the least disturbed in the whole area. Annual rings indicates little disturbance in the past, only single trees show some small increase in growth at different times.
Plot 10
This stand lies on the top of the esker so the ground is stony, and the same phenomena here as in area 4 with bilberries’ growing in patches. The oldest tree drilled in this plot is about 190 years old. It is not the mean age but many of the trees are about that age. This area is however multi-layered with trees in all ages so the mean age is about 144 years. The tree density is about 7 trees per 10 m², not included seedlings that is quite abundant in patches. The seedlings consists of most pine but also spruce and they are all older than 30 years even though they are less than 1,5 meter high. Tree stumps are scarce with less than one per 10 m². Almost all stumps are degraded. Annual rings show no signs of increased growth indicating that cutting have been minimal in this area.

Plot 11
Most of the trees in this stand are about 30 years old. The oldest one located about 40 years. Tree density is about 12,2 trees per 10 m². Tree stumps have a quite high frequency with more than 5 stumps per 10 m², not included small stumps from the most resent thinning. Both fresh and old dead woody debris is spread around the area, some are more decomposed branches probably left behind after logging in the past and some are thinner trunks from more resent thinning. Annual rings show no signs of increased growth indicating that all trees drilled started growing after the last cutting, except the last thinning whom has been done in the past year or two.

Plot 12
This stand has some of the oldest trees; most trees are about 150 years old. The oldest tree drilled is at least 185 years old. The frequency of smaller trees are quite high, but the branches ends so high that it is impossible to determine their age by counting branches, other than certify that they are old. Tree density is quite irregular with an average at 7,1 trees per 10 m². Tree stumps are scarce with just more than 1 stump per 10 m². The annual rings only show some slight increase in growth about 70-80 years ago. Even though there are quite large spots with no trees and almost no vegetation, and no signs of discrimination of seedlings by cutting there are few seedlings trying to establish.

Plot 13
This stand has recently been thinned. The density of trees is less than 6 trees per 10 m². Most trees are in the age of 70 years old and the oldest tree located about 110 years old. Tree stumps is quite frequent with more than 5 stumps per 10 m², about half from the most resent thinning and the rest in various stages of degradation. Annual rings show an increase in growth at several occasions from 40 to 60 years ago. Fresh dead wood is quite abundant and occasionally in stacks left behind after the most resent thinning.

Plot 14
Most trees in this area are about 75 years old and the oldest tree drilled about 100 years old. The tree density is variabel with an average at about 9,5 trees per 10 m². The frequency of tree stumps is less than 3 per 10 m², and all are quite degraded. Annual rings show an increase in growth about 40 years ago. There are several spots in the area with no ground vegetation and without trees and no sign of discrimination of seedlings by cutting; still there are no establishing seedlings.
Plot 15
This is a narrow forest stand on the south side of the esker, bordering an older forest on top of the esker and a mixed forest downhill the esker to the south. All trees are in the approximately same age, about 50 years. The oldest tree located was about 58 years old. Tree density is high with more than 22 trees per 10 m². The frequency of tree stubs is quite low with about 3,5 stumps per 10 m², and all are quite degraded. In parts of the stand there are a few smaller newer stumps after a resent thinning. Annual rings show no sign after thinning indicating that all trees in the stand grew up after the last cutting.

Plot 16
In this stand some logging activity has recently been done, but the tree density is still quite high with about 7,4 trees per 10 m², and older trees are there are still quite abundant. Most trees about 100 years and the oldest tree located about 150 years. The tree rings indicate some growth increase about 100 years ago. Most tree stumps are fresh from the recent thinning with a frequency at about 5 stumps per 10 m².

Plot 17
All trees in this area are between 40 and 60 years old. Tree density is high with about 22,7 trees per 10 m². The frequency of tree stumps is quite low with just above 2 stumps per 10 m². All trees are thin so the age was determined by counting branches and no drilling was done, but the low frequency of tree stumps indicates that there has been no resent logging activity in this area. One stump on the edge of the area showed sign of fire so one plausible reason for why there are so few stumps and no older trees can be that there has been a fire in the stand.

Plot 18
This is a middle aged forest with all trees in an age of approximately 60 years. Some older trees have been left behind close to an old execution site but they didn’t fall within any of the study plots so they were not age determined. The oldest tree drilled was about 60 years. The stand has recently been thinned. The tree density was still high compared with the mean in the whole area with just above 10 trees per 10 m², and the frequency of tree stumps was also quite high with almost as many stumps as trees, most new from the latest thinning but also some older ones from an earlier thinning. Annual rings show an increase in growth about 40 and 20 years ago.

Plot 19
A young forest with most trees about 20-30 years. The oldest tree located is about 40 years old. Compared with the other younger areas, tree density is quite low in this area with less than 10 trees per 10 m². Tree stumps are almost as frequent as trees, with more than 9 per 10 m², both bigger older ones probably from the clear cutting of the area, bigger newer ones assumingly from cutting down seed trees and smaller fresh ones from a resent thinning. Only a couple of trees were drilled to determine logging activities and the annual rings showed an increased growth about 20 years ago which is probably due to cutting of seed trees. Dead wood is shattered around the area, both old and new. There are deep tracks in the ground after a forwarder.
Appendix B
Species List of all fungal species found in the area:

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Appendix C

**Mycorrhiza**

Mycorrhiza comes from the Latin words mykos, meaning fungi and riza meaning root, in other words “fungus root”. Mycorrhiza is a symbiotic relationship between vascular plants and fungi, were the fungi among other things mediates important nutrients like phosphorus, nitrogen and minerals to the tree and facilitates uptake of water. In return the fungi are supplied with photosynthetically fixed carbon from its host plant. There are several different types of Mycorrhiza and one of the most common is ectomycorrhiza which is the type of mycorrhiza sheared between trees and *Hydnellum* species. This type of mycorrhiza is generally characterised by two main features: a mantle of fungal tissue surrounding the root tip; and a Hartig net composed of a hyphal network between the epidermal and cortical cells of the root. It is in this interface the nutrient exchange takes place. Most mycorrhizal fungi are obligate symbionts of vascular plants and can’t survive without the host plant. (Smith and Read 2008)