



Gap-edge Effects on Seedling Growth, N Uptake and Associated Ectomycorrhizal Fungi

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Degree project • 60 credits
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Master of Science in Forestry
2024:08 • ISSN 1654-1898
Umeå 2024



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Credits: 60 credits

Level: A2E

Course title: Master's thesis in Forest Science, A2E - Forest Ecology and
Management

Course code: EX0957

Programme/education: Jägmästarprogrammet

Course coordinating dept: Department of Forest Ecology and Management

Place of publication: Umeå

Year of publication: 2024

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Title of series: Examensarbeten / SLU, Institutionen för skogens ekologi och skötsel

Part number: 2024:08

ISSN: 1654-1898

Keywords: Regeneration, Ectomycorrhiza, Nitrogen, Gap-edge effect, Boreal, Fungi

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Abstract

Ectomycorrhizal fungi (EMF) form symbiotic relationships with dominating trees in boreal forest ecosystems that provide the trees with necessary nutrients and water in exchange for photosynthetically derived carbon. This makes them important to both forest productivity and ecosystem functioning, especially as forestry is seeking to decrease clear-cuts. Yet the question of regeneration in alternative types of forest management in northern boreal Sweden is still not solved. This thesis combines amplicon sequencing and isotopic modelling to determine the impact of belowground competition on seedling growth and N uptake in connection to their ectomycorrhizal community in the gap-edge zone where seedlings compete with mature trees for resources. Results indicate that belowground competition is an important determining factor. Excluding seedlings from competition by using barriers in the soil increased their growth and nutrient uptake. They also had a different ectomycorrhizal community on their roots with more short-growing genera on excluded seedlings. This puts the focus on the belowground competition mediated through the mycelium of fungi, showing potential future research that can answer how to get the best regeneration using alternative forest harvesting methods in boreal Sweden.

Keywords: Regeneration, Ectomycorrhiza, Nitrogen, Gap-edge effect, Boreal, Fungi

Table of contents

List of figures	5
1. Introduction.....	7
2. Methods.....	10
2.1 Site location and experiment description.....	10
2.2 DNA extraction and amplicon sequencing	10
2.3 Isotope analysis	12
2.4 Statistical analysis	13
3. Results	14
3.1 Seedling growth	14
3.2 Nitrogen uptake.....	15
3.3 Mycorrhizal community	18
4. Discussion	21
5. Conclusion	24
References.....	25
Popular science summary.....	30
Acknowledgements.....	31

List of figures

- Figure 1 Overview of the site in Torrböle illustrating the experimental design. Clear-cut on a sandy pine-heath with intact forest dominated by *Pinus sylvestris* L. on the west and east side. Three treatments; exclusion, cut and control were replicated 5 times at each distance (-10, 0, 4, 8, 20 meters from forest edge) along each transect. Each treatment combined a *P. sylvestris* seedling and a *Picea abies* (L.) H.Karst. 11
- Figure 2 Total dry-weight of seedlings split by treatment and species. Distance denotes distance from forest edge (positive distance into the clear-cut). Letters indicate significant difference of means ($\alpha=0.05$) using Tukey HSD. 15
- Figure 3 Relative growth compared to control (Dry-weight/Dry-weight of corresponding control seedling) at different distances from the edge of the mature stand. Error bars denote standard error. 16
- Figure 4 $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$ isotopic ratio expressed using the atmospheric N scale) for each treatment and distance. Left shows *P. sylvestris* and right shows *P. abies*. 16
- Figure 5 Nitrogen concentration in seedling needles. Letters indicate significant difference ($\alpha=0.05$) following a post-hoc test, using a multiple t-test between each treatment within each distance. 17
- Figure 6 Nitrogen uptake since planting in milligrams split by pine and spruce. Letters denote significantly different means ($\alpha=0.05$) using TukeyHSD post-hoc test. 17
- Figure 7 Shannon Diversity Index of ectomycorrhizal genera on the roots of *P. sylvestris* and *P. abies* for the three treatments. Colours denote distance from forest edge. 19
- Figure 8 Relative abundance of the 12 most abundant ectomycorrhizal fungi (EMF) genera by treatment and distance. All other EMF have been grouped as Other. 19
- Figure 9 Principle Coordinate Analysis (PCoA) of ectomycorrhizal community composition in *P. sylvestris* and *P. abies*. Colours denote treatment (Control=red, Cut=green, Excluded=blue) and shapes denote distance from forest edge. ...20
- Figure 10 Relative abundance of ectomycorrhizal exploration types as defined by Agerer (2001) using the FungalTraits database. Exploration types have been further

grouped into long distance, short distance with regards to life-history similarities. Long distance includes long-distance and medium-distance fringe. Short distance includes all short distance variants and medium-distance smooth. A clear pattern emerges where exclusion of competition from surrounding trees promotes establishment of short distance exploration types.
..... 20

1. Introduction

The factors that determine successful forest regeneration remain a central question for forest productivity in northern boreal Sweden. Currently, it is based on manual planting of clear-cut forests and soil scarification through either mounding or harrowing. These methods have been developed since the late 19th century and subsequently applied across the majority of the productive forests (Lundmark et al. 2013). Due to this, Swedish forests are well suited for wood and biomass production but lack the ability to support biodiversity compared to other boreal countries (Naumov et al. 2018). New forestry methods, such as continuous cover forestry (CCF), are used to combine production with biodiversity and are gaining global traction following a recognition of the ecological consequences of current methods and a changing societal view of the value of the forest (Puettmann et al. 2015). A focus within the European Union on forestry further highlight an increased awareness on biodiversity in forest management (European Commission 2021). There are still systemic barriers within Sweden that limit the adoption of CCF (Hertog et al. 2022), but an increased international and domestic awareness and valuation of the consequences of biodiversity in clear-cut forestry likely will lead to increased adoption of CCF in Sweden.

Apart from systemic barriers to adoption, ecological barriers exist, especially in northern boreal Sweden. Forest management systems such as CCF require regeneration under a partial canopy in close competition with dominant trees. Established canopies have historically shown poor regeneration, and this has been well-known for over 100 years in northern boreal Sweden (Björkman 1945; Hagner 1963) and could become a challenge in adopting CCF. Clear-cutting and manual planting was introduced as an answer to the residual stands of Scots pine left after high-grading, commercial cutting of trees above a certain diameter, which exhibited poor regeneration in an effort to ensure regeneration as to ensure compliance with regulations requiring logging not to have a negative impact on the long-term productivity of the forest (Lundmark et al. 2013). So, although the effect of competition with mature trees has been recognised, the reasons behind it are still unknown.

Growth of seedlings are characterized by their competition for light, water and nutrients (Bowman et al. 2017). Historically, aboveground competition, light and biotic influences, have been considered the most important factors governing the

success of regeneration (Axelsson et al. 2014), however studies from early to mid-1900's (Björkman 1945; Kuuluvainen & Ylläsjärvi 2011) and later (Coomes & Grubb 2000; Axelsson et al. 2014; Högberg & Högberg 2022) show the importance of belowground nutrient competition in directing seedling regeneration patterns. The lateral extension of roots from mature trees in boreal forests is around 5 meters (Bishop 1962; Taskinen et al. 2003; Johnsen et al. 2005) which was confirmed by isotopic analyses to corresponds with the lateral extent of nutrient uptake activity (Gottlicher et al. 2008; Henriksson et al. 2021; Lutter et al. 2021).

For their nitrogen (N) uptake, boreal forest trees largely rely on ectomycorrhizal fungi (EMF). EMF form symbiotic relationships with trees in boreal forest ecosystems that provide the trees with necessary nutrients and water in exchange for photosynthetically derived carbon (Smith & Read 2008). This makes them important to both forest productivity and ecosystem functioning. However, although acting as a symbiont, EMF have also been shown to cause immobilization of belowground N in northern boreal conditions (Näsholm et al. 2013).

Despite the increasingly recognised importance of EMF, the research has primarily developed outside of the ecological mainstream (Kennedy 2010), only lately moving towards a functional trait-based approach (Camenzind et al. 2024) due to the great diversity within fungal communities and the immense challenge of in-vivo measurements. Trait-based approaches are based on the concept of limited resources meaning that a species cannot be at optimal functioning capacity in all aspects and conditions (Kneitel & Chase 2004). Concepts such as the Grime's C-S-R triangle are based on this approach (Grime 1977). Applying these concepts to fungal ecology could further advance our understanding of ecosystem function and nutrient competition in boreal forest ecosystems.

EMF communities contain a great interspecific diversity, often with a few dominant and several rare species (Tedersoo et al. 2012a; Kyaschenko et al. 2017). Different species differ in their ability to exploit different inorganic (Hobbie & Högberg 2012) and organic N sources while showing strong plasticity in phenotype expression depending on abiotic conditions (Smith & Read 2008). The most important explanatory variable for their ability to exploit different N sources, their enzymatic ability, is the abundance and morphology of the extraradical mycelial system (Tedersoo et al. 2012b). Agerer (2001) defined several "exploration types" that can be likened to foraging strategies through the morphology of fungal extraradical mycelium. These range from contact, to short-, medium- and long-distance types. Medium- and long-distance types appear similarly able to exploit organic N sources and lower carbon-use efficiency through creation of rhizomorphs that lessen their competitive ability in disturbed systems such as clear-cuts (Lilleskov et al. 2011). Short- and contact exploration types are more common in disturbed soils due to faster regeneration of their mycelium. This largely follows the limited resource concept where species can either spread quickly or are highly

competitive. Clear-cut harvesting has been shown to greatly decrease the EMF biodiversity in the soil (Sterkenburg et al. 2019) and to change the community composition leading to changes in functional diversity (Kyaschenko et al. 2017).

This study aims to investigate the ecological barriers to the introduction of CCF forestry methods in the northern boreal zone of Sweden. The main question to answer is whether belowground competition close to mature trees influences the EMF community composition of planted seedlings and in turn how this affects the N uptake and growth of the seedlings. To do this, seedlings of *Pinus sylvestris* L. and *Picea abies* (L.) H.Karst were planted at various distances from a clear-cut edge, with varying exclusion treatments from surrounding mycelium and other roots. These seedlings were grown with ¹⁵N in the greenhouse before outplanting, allowing isotope analysis of seedling N uptake and investigation of biomass growth. By combining this isotope analysis with amplicon sequencing of the roots could reveal their associated EMF community composition and test the following hypotheses:

- I. Removal of competition through root exclusion results in higher seedling growth and N uptake in the forest. In the clear-cut, root exclusion should have no effect compared to control.
- II. Ectomycorrhizal fungi species diversity and richness will decrease with increasing distance from clear-cut edge in seedlings in competition with larger trees.
- III. Seedlings with a higher relative abundance of associated EMF of the long-distance exploration type should take up less N.

2. Methods

2.1 Site location and experiment description

Site location is Torrböle, Västerbotten, Sweden (N63.7E19.6) on a pine heath on sandy sediment soil. Field vegetation is dominated by lichens (*Cladonia* spp.) and ericaceous dwarf shrubs (*Vaccinium vitis-idaea* L., *Calluna vulgaris* (L.) Hull). The clear-cut was scarified using mounding and planted with pine in 2020, three years before the labelled study seedlings were planted in the field. The labelled seedlings were marked with ^{15}N while grown in the Skogforsk nursery in Sävar for four months before planting. Planting was done at the start of the growth season in 2022. Three treatments were implemented; control, cut (a shovel was used to cut into the soil around the seedlings, severing any mycelial or root connections), and exclusion (seedlings were planted in containers with an opening in the bottom that was covered by a nylon mesh, 45 μm pore size (Sintab Product AB, Oxie, Sweden), for drainage). Each treatment was repeated five times at five distances (-10, 0, 4, 8, 20 meters from forest edge) along 8 transects. Four on the west side and four on the east side of the clear-cut (Figure 1).

Seedlings from one of each treatment at each distance and transect were destructively harvested in September 2023 by manually digging them up with a shovel, maintaining as much of the root system as possible. Seedlings were cut into shoot and root, just above the root collar, at the time of harvest. Fresh weight was taken directly in the field. From each root sample, five to six of the individual roots that were deemed representable for all seedling roots were put into falcon tubes and stored on dry ice for transport before being stored at -20°C for up to 3 months.

The shoots were put into an oven at 60°C for two weeks, before being stored at room temperature for up to two and a half months. Dry weights were taken of roots and shoots.

2.2 DNA extraction and amplicon sequencing

DNA extraction was done using Qiagen Plant Pro Kit following manufacturer's instructions with negative controls. Samples were stored at -70°C and thawed on

ice before amplification. Amplification of the ITS2 (Internal Transcribed Spacer 2) region was run in triplicates, each containing 12.5 μ l KAPA HiFi HotStart Mix, 2.5 μ l of ITS4 [10 μ M] and gITS7 [10 μ M] each (described in White et al. (1990) and Ihrmark et al. (2012), each primer mixed equally to final concentration), 5ng of DNA sample [5ng/ μ l] or 1ng of a fungal mock community [0.5ng/ μ l] for positive control or MilliQ water for negative control, and then diluted with MilliQ water to a final volume of 25 μ l. We used the same mock community as published in Haas et al. (2018). PCR amplification used the following protocol: Initial polymerase activation at 95°C for 3 minutes, then denaturation at 98°C for 20 seconds, annealing at 58°C for 15 seconds, extension at 72°C for 20 seconds, repeated from denaturation 30 times. Final extension at 72°C for 2 minutes. Triplicates were pooled and 25 μ l of PCR product (amplicon) of each pooled sample plus 5 μ l of 6X dye was analysed on agarose gel 1.5-2% in TAE buffer.

PCR products were sent for sequencing at the National Genomics Institute (NGI) in Solna, Stockholm. Further clean-up and barcoding PCR steps were performed on-site by NGI lab staff. Samples were pooled and sequenced using an Illumina MiSeq machine with 600 cycles, resulting in 300bp paired-end reads.

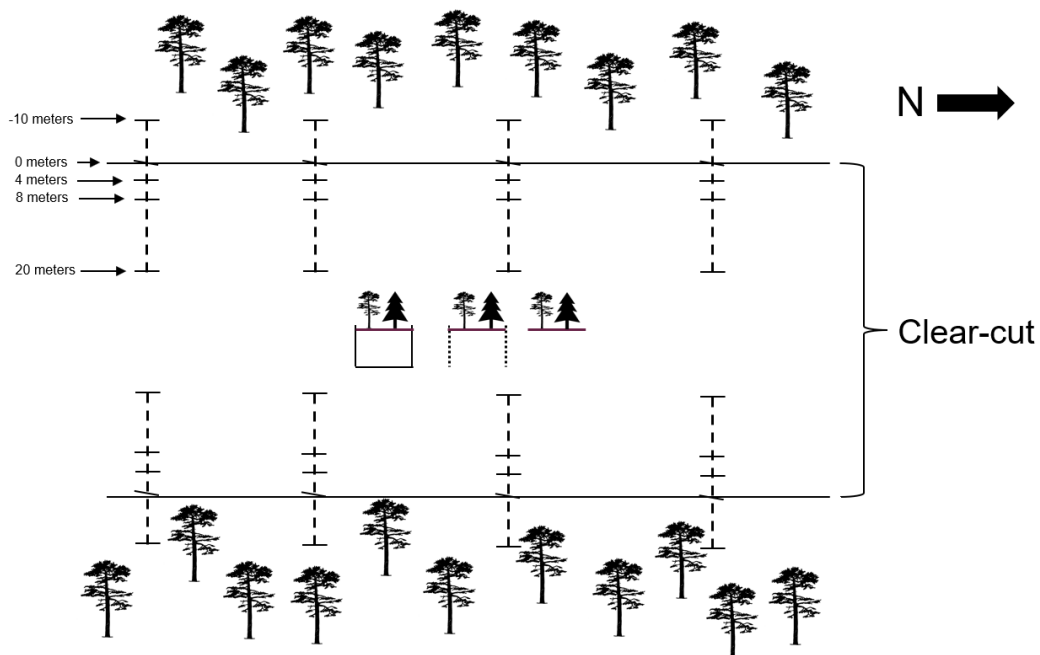


Figure 1 Overview of the site in Torrböle illustrating the experimental design. Clear-cut on a sandy pine-heath with intact forest dominated by *Pinus sylvestris* L. on the west and east side. Three treatments; exclusion, cut and control were replicated 5 times at each distance (-10, 0, 4, 8, 20 meters from forest edge) along each transect. Each treatment combined a *P. sylvestris* seedling and a *Picea abies* (L.) H.Karst.

Demultiplexing and initial quality filtering of sequencing data were performed at NGL.

Sample fastq files were pre-processed using dada2 (v. 1.31), unless stated otherwise (Callahan et al. 2016). Reads were pre-filtered to remove Ns, and subsequently cut using primer sequences and cutadapt (v. 3.4) (Martin 2011). The reads were quality filtered and trimmed using parameters ‘maxN = 0, maxEE = c(4,4), truncQ = 2, minLen = 50, rm.phix = TRUE’. Filtered reads were used for error learning, dereplication, denoising and merging of forward and reverse reads with a maximum mismatch of 1 base. Chimera removal was performed using the ‘consensus’ method. Resulting amplicon sequence variants (ASVs) were clustered into operational taxonomic units (OTUs) using swarm (v. 3.1.3) with parameter d set to 3 (Mahé et al. 2021). Taxonomy was assigned to cluster centroid sequences using the naïve Bayesian classifier implemented in dada2, with the UNITE database (All eukaryotes, release 10.0) used as reference (Abarenkov et al. 2024). Non-fungal OTUs were removed before further analysis. Fungal functional guilds were identified using FungalTraits at the genus level (Pöhlme et al. 2020), a database of traits that has shown good precision (Tanunchai et al. 2023). Of the identified EMF OTUs, samples with less than 4000 reads (4 samples) were removed to avoid skewing comparisons.

Diversity was calculated using the Shannon Diversity Index that indicates the evenness of species in a community. Higher indicates a higher diversity, with 0 if there is only one species in a sample. This is calculated as:

$$\text{Shannon Diversity Index} = -\sum p_i * \ln(p_i)$$

p_i = The proportion of the community consisting of species i

Richness was calculated as the sum of OTUs in each sample following rarefaction using the vegan R package (version 2.6-6.1, Oksanen et al. 2024) rrarefy function with sample size set to the richness of the sample with the lowest richness.

2.3 Isotope analysis

Samples of the dried needles and roots were ground into fine powder in a ball mill and weighed into tin capsules (5mg±10% per sample). Isotope analysis was done using an isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Bremen, Germany) and an elemental analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) (Werner et al. 1999).

For further analysis, an isotopic mixing model (Fry 2006, see Equation 1 and 2) was utilised. Due to the different known proportion of nitrogen taken up by the plant in the greenhouse and in the field, it is possible to calculate the proportion of each source using the following equation:

$$f1 = (\delta_{SAMPLE} - \delta_{SOURCE2}) / (\delta_{SOURCE1} - \delta_{SOURCE2}) \quad \text{Equation 1}$$

f1 = fraction of N from the greenhouse

δ_{SAMPLE} = $\delta^{15}\text{N}$ in sample

$\delta_{SOURCE1}$ = $\delta^{15}\text{N}$ of seedling needles after greenhouse labelling

$\delta_{SOURCE2}$ = seedling needle natural abundance $\delta^{15}\text{N}$

Using this, the fraction of N in each seedling that had been taken up in the field could be calculated as a percentage of the dry-weight.

Recognizing that $f1+f2=1$, where f2 is the fraction of N from the field, the relative amount of N taken up in the field could be calculated for each sample using Equation 2.

$$N \text{ from field [g]} = f2 * \text{total N [g]} \quad \text{Equation 2}$$

Comparing initial ^{15}N amount to sampled ^{15}N amount was done by taking the isotopic amount fraction $^{15}\text{N}/(^{14}\text{N} + ^{15}\text{N})$ (calculated from $\delta^{15}\text{N}$ using $R_{\text{ref}} = ^{15}\text{N}/^{14}\text{N} = 1/272$) times the average N molar mass in the sample. The average N molar mass was calculated as the atomic fraction of ^{15}N times the molar mass of 15 adding the atomic fraction of ^{14}N times the molar mass of 14.003.

2.4 Statistical analysis

Statistical analysis was done with R version 4.3.3 (R Core Team 2024) using packages *dplyr* (version 1.1.14, Wickham et al. 2023) and *reshape2* (version 1.4.4, Wickham 2007) for data manipulation.

Statistical hypothesis testing was done using two-way ANOVA using the following model:

$$aov(\text{response} \sim \text{Treatment} * \text{Distance} + \text{Transect})$$

Where *response* is the desired response variable including dry-weight of the seedlings, nitrogen uptake, diversity and richness. Results from the ANOVA was checked using the *car* package (version 3.1-2, Fox & Weisberg 2019). In case of significance ($\alpha=0.05$) a post-hoc test was done using *emmeans* (version 1.10.1, Lenth 2022). All post-hoc tests used were done with Tukey HSD, except for testing relative growth that used a Dunnett test to compare with control. All graphs were plotted using the *ggplot2* R package (version 3.5.0, Wickham 2016), *stringr* (version 1.5.1, Wickham 2023) and *multcomp* (version 1.4-25, Hothorn et al. 2008).

3. Results

At the time of planting, spruce seedlings had an average dry-weight of 0.39 g and pine seedlings an average dry-weight of 0.33 g. Pine had a slightly lower N concentration of 1.7% compared to spruce at 2%. Interestingly, pine had a lower average N concentration (1.67%) in the needles than spruce (2.19%). Seedling isotopic labelling was successful with both species showing a high $\delta^{15}\text{N}$ isotopic ratio in both roots and shoots (pine root: 2001‰, pine shoot: 2309‰; spruce root: 1871‰, spruce shoot: 2390‰)

3.1 Seedling growth

Seedlings in the exclusion treatment were significantly larger than seedlings in the other treatments having a mean of $3.90 \text{ g} \pm 2.04 \text{ g}$ dry-weight for *P. abies* and $5.63 \text{ g} \pm 3.2 \text{ g}$ dry-weight for *P. sylvestris* ($p < 0.0001$, ANOVA). Control and cut treatments did not differ significantly from each other at any distance from the forest edge. Seedlings in the two treatments grew slightly better with increasing distance from forest edge, starting around 4 to 8 meters from the edge for both species. The distance from forest edge did not affect the growth of excluded seedlings.

Seedlings in the cut treatment did not grow significantly better than controls. Excluded seedlings grew significantly better than the other treatments. On average, pine seedlings in the excluded treatment grew 300% ($se=42.8$) better than controls, though the difference decreased with an increasing distance from the edge. Spruce seedlings in the excluded treatment had a smaller difference between control and excluded seedlings than pine at 232% ($se=34.2$) higher dry-weight (Figure 3).

Seedlings that were excluded from competition by being planted in containers grew better and had taken up more N to their needles. This was true for both *P. sylvestris* and *P. abies* and at all distances. This effect was most pronounced in the forest, where both species on average grew more than 300% better than the control seedlings. As distance into the clear-cut increased, the positive exclusion effect decreased but this effect was not statistically significant and exclusion treatment resulted in a more than 100% higher total dry-weight at all distances for spruce and 200% for pine.

3.2 Nitrogen uptake

During the time since planting, seedling $\delta^{15}\text{N}$ decreased showing uptake of N in the field. All the initial ^{15}N label remained in the seedlings after 2 growing seasons, indicating no loss of N from the seedlings. Root carbon (C) concentration results indicated contamination of soil minerals in the samples leading to N analysis being focused on needle N.

The results from the N isotope analysis indicate N limitations in seedlings exposed to competition within and close to the forest edge. Needles of excluded seedlings had a significantly higher mean N concentration (pine = $1.21\% \pm 0.35\%$, spruce = $1.29\% \pm 0.38\%$) compared to the other treatments that were below 1%. However, across distances, pine needles only had a significantly higher N concentration in the forest (-10 meters from edge). In spruce, N concentration was significantly higher than controls within the forest and edge zone. Excluded seedlings had a significantly higher N concentration in the forest and at 4 meters from the edge, still within the edge zone (Figure 5).

By using an isotopic mixing model (Equation 1) and the seedling dry-weight, the total N uptake since planting could be calculated (Figure 6). This gives a direct comparison of the fierceness competition for N in the different treatments. Both pine and spruce needles in the excluded treatment had taken up significantly more N than the other treatments.

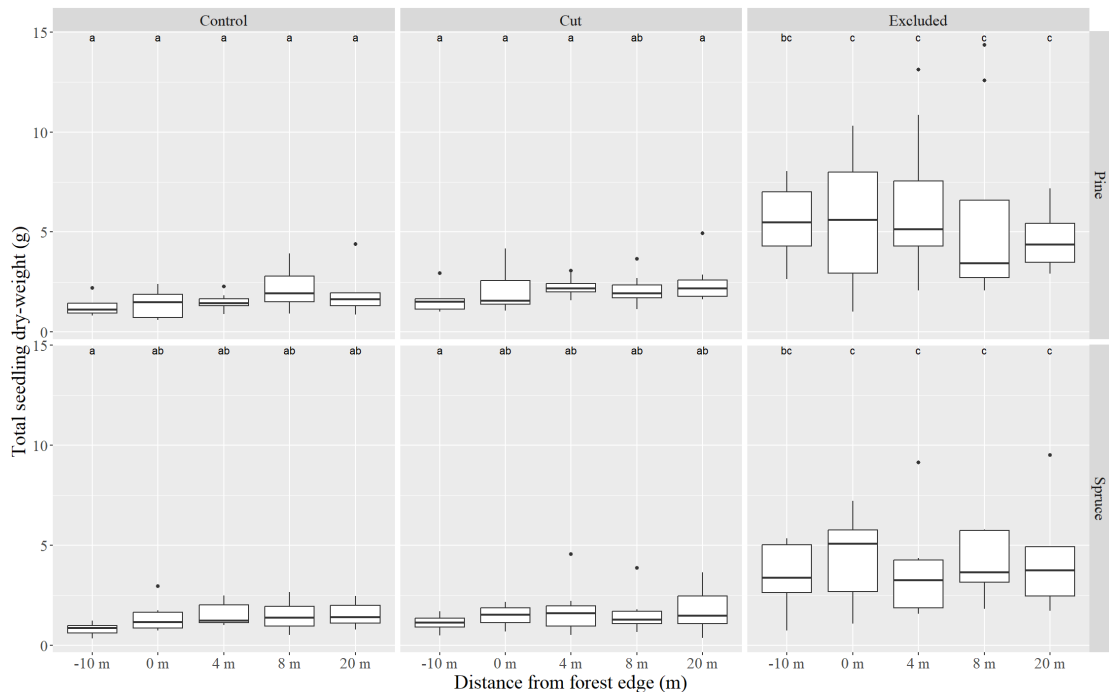


Figure 2 Total dry-weight of seedlings split by treatment and species. Distance denotes distance from forest edge (positive distance into the clear-cut). Letters indicate significant difference of means ($\alpha=0.05$) using Tukey HSD.

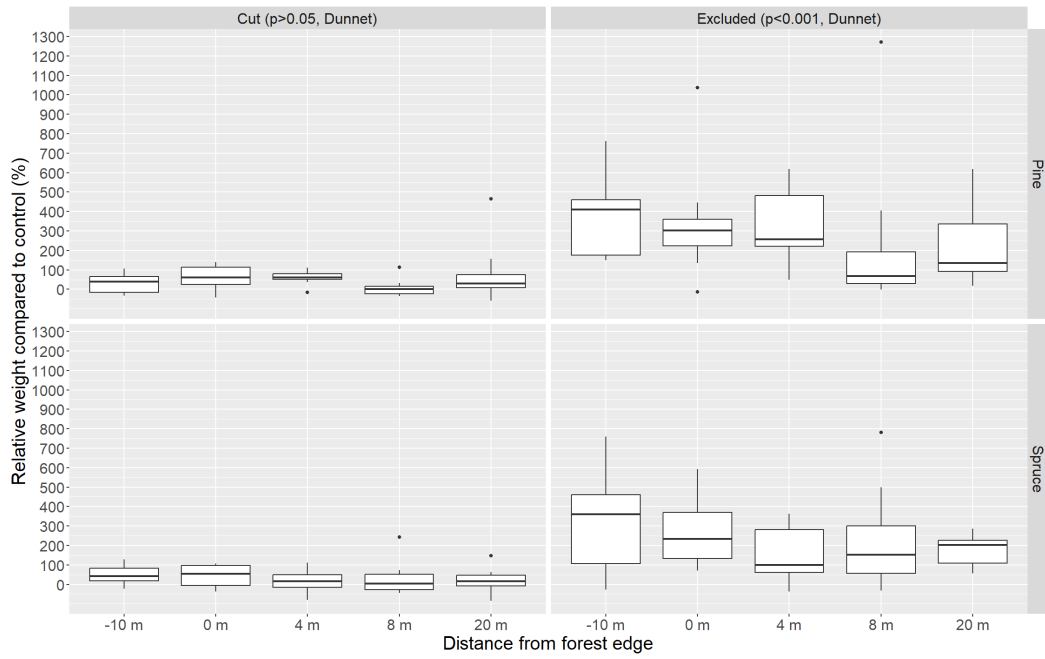


Figure 3 Relative growth compared to control (Dry-weight/Dry-weight of corresponding control seedling) at different distances from the edge of the mature stand. Error bars denote standard error.

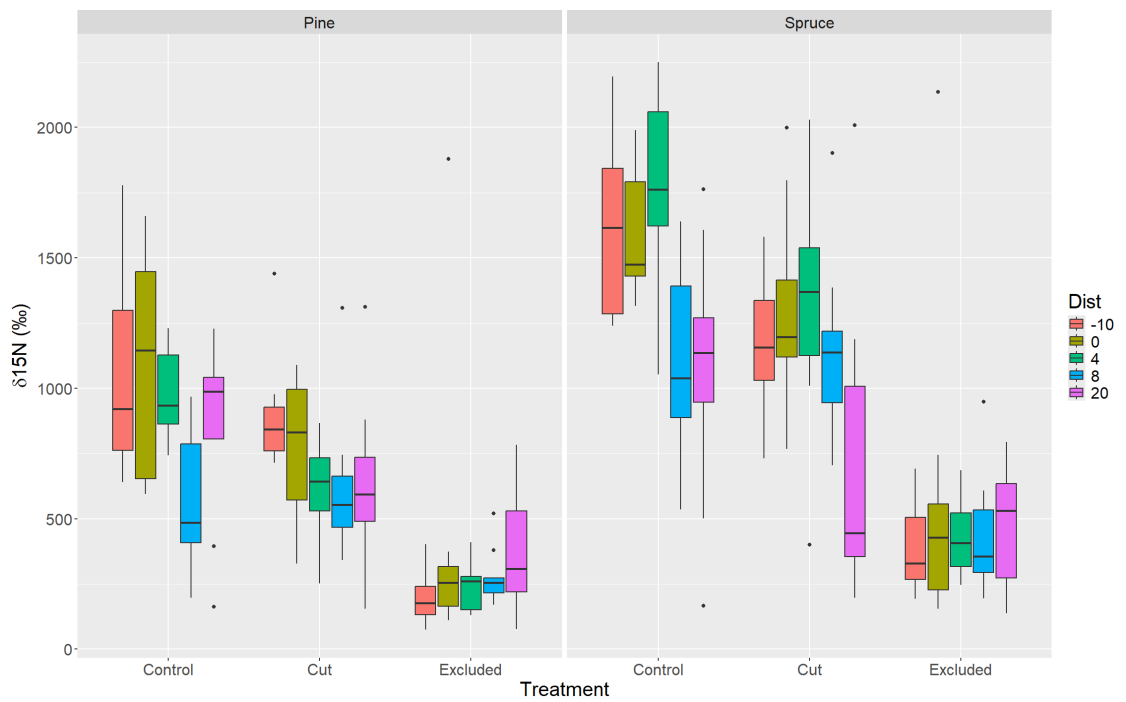


Figure 4 $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$ isotopic ratio expressed using the atmospheric N scale) for each treatment and distance. Left shows *P. sylvestris* and right shows *P. abies*.

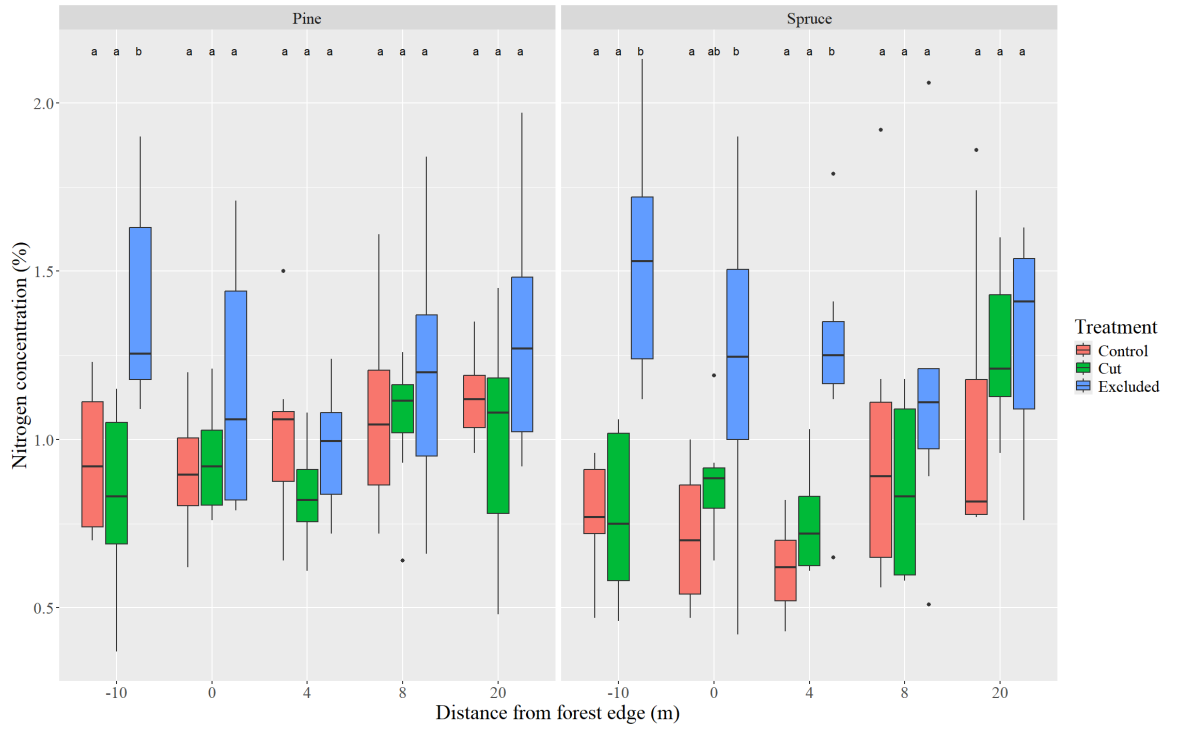


Figure 5 Nitrogen concentration in seedling needles. Letters indicate significant difference ($\alpha=0.05$) following a post-hoc test, using a multiple t-test between each treatment within each distance.

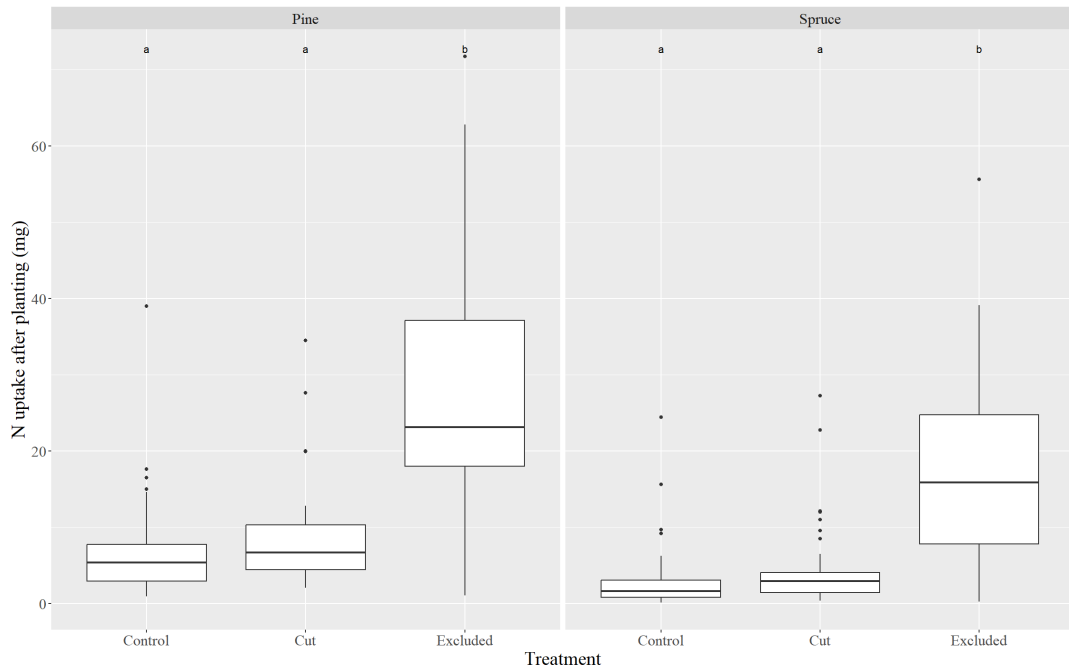


Figure 6 Nitrogen uptake since planting in milligrams split by pine and spruce. Letters denote significantly different means ($\alpha=0.05$) using TukeyHSD post-hoc test.

3.3 Mycorrhizal community

ITS2 amplification and analysis was used to identify fungal communities on the samples. After quality control, a total of 7994297 sequences remained across 936 OTUs. Of these, 596 were able to be identified at the genus level using the FungalTraits database (Põlme et al. 2020). Out of these, 121 OTUs were identified as ectomycorrhizal by their primary lifestyle divided into 33 genera. The most common genera were *Cortinarius* with 24 OTUs, followed by *Piloderma* at 9 OTUs.

Diversity measured using Shannon's Diversity Index could not be explained by distance from forest edge. Both species had a significantly lower diversity in EMF community composition in the exclusion treatment compared to the other treatments. Diversity for both species was highly variable. Richness also did not show any significant ($p < 0.05$) change with distance. Spruce did however have a higher species richness than pine, though this was not able to be tested statistically.

EMF community composition showed clear patterns across treatments and seedlings (Figure 8). In the exclusion treatment, *Thelephora* sp. was the most abundant for both species. *Thelephora* sp. continued to be the most abundant in the cut treatment at 20 meters for both species. In pine, *Suillus* sp. was most abundant in the forest and edge zone together with *Piloderma* sp. in the cut treatment. In the pine controls, *Suillus* sp. increased in abundance with increasing distance from forest edge. In the edge zone and forest, *Rhizopogon* sp. and *Piloderma* sp. were also relatively abundant but decreased in abundance in the clear-cut where *Thelephora* sp. increased in abundance. For spruce, species abundance on controls and cut treatment were more even with no genera being dominant. However, *Thelephora* sp. became more dominant in the clear-cut.

Principle coordinate analysis showed clear grouping for samples in the exclusion treatment and samples at 8 and 20 meters (Figure 9). For the pine samples, there is also a second, separate cluster however with some samples spread across the graph. Spruce did not show any second cluster but had a large variance in EMF community composition between samples that were not excluded from competition.

Short-distance exploration types dominated the samples outside of the competition zone (8 and 20 meters from forest edge) for both species (Figure 10). Within the competition zone (-10, 0, and 4 m from forest edge) there was a difference between the species. Pine had a higher relative abundance of long-distance exploration types for control seedlings at all distances, whereas cut seedlings had a lower relative abundance of long-distance exploration types in the clear-cut. Spruce had a lower relative abundance overall of long-distance exploration types and had a higher relative abundance of short-distance exploration types.

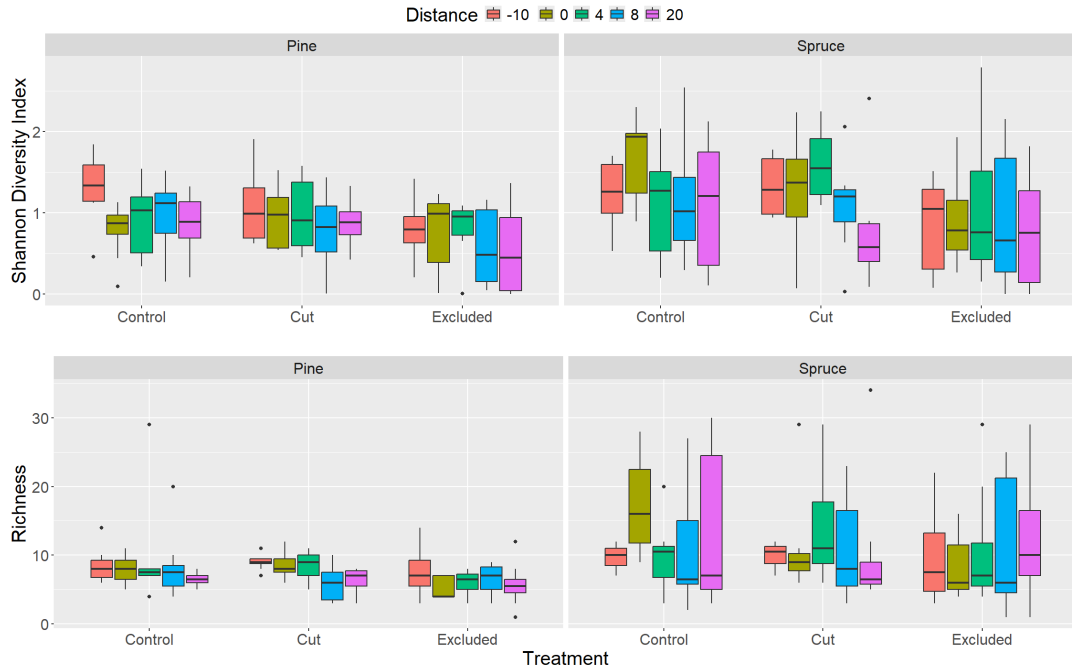


Figure 7 Shannon Diversity Index of ectomycorrhizal genera on the roots of *P. sylvestris* and *P. abies* for the three treatments. Colours denote distance from forest edge.

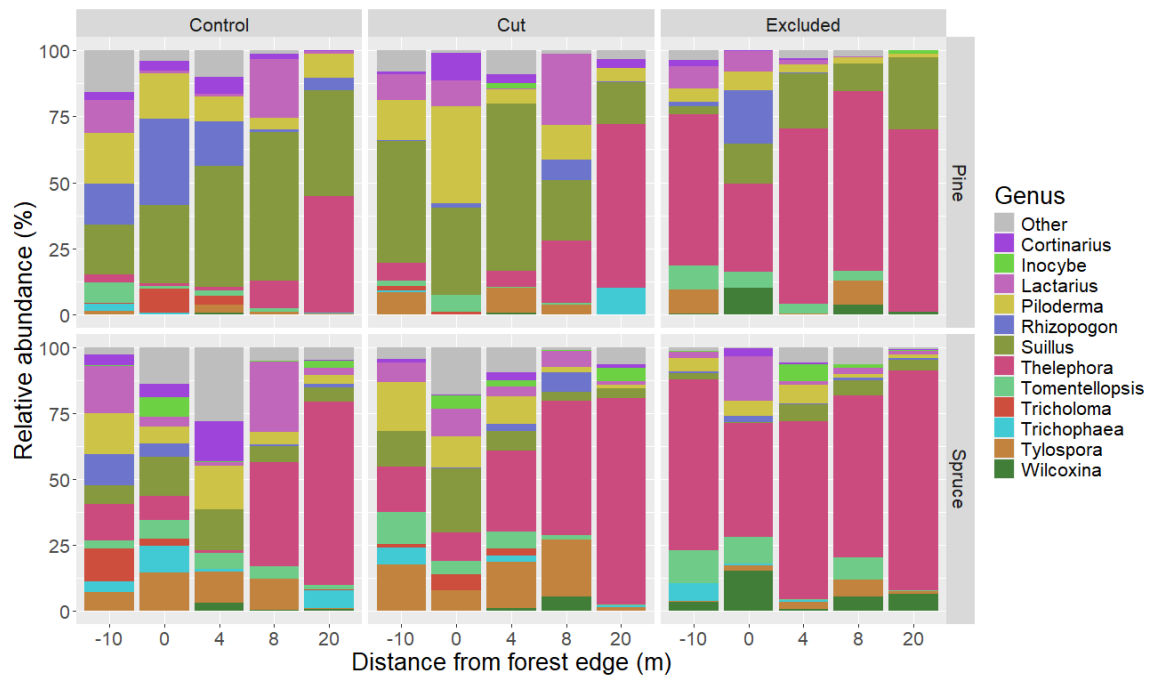


Figure 8 Relative abundance of the 12 most abundant ectomycorrhizal fungi (EMF) genera by treatment and distance. All other EMF have been grouped as *Other*.

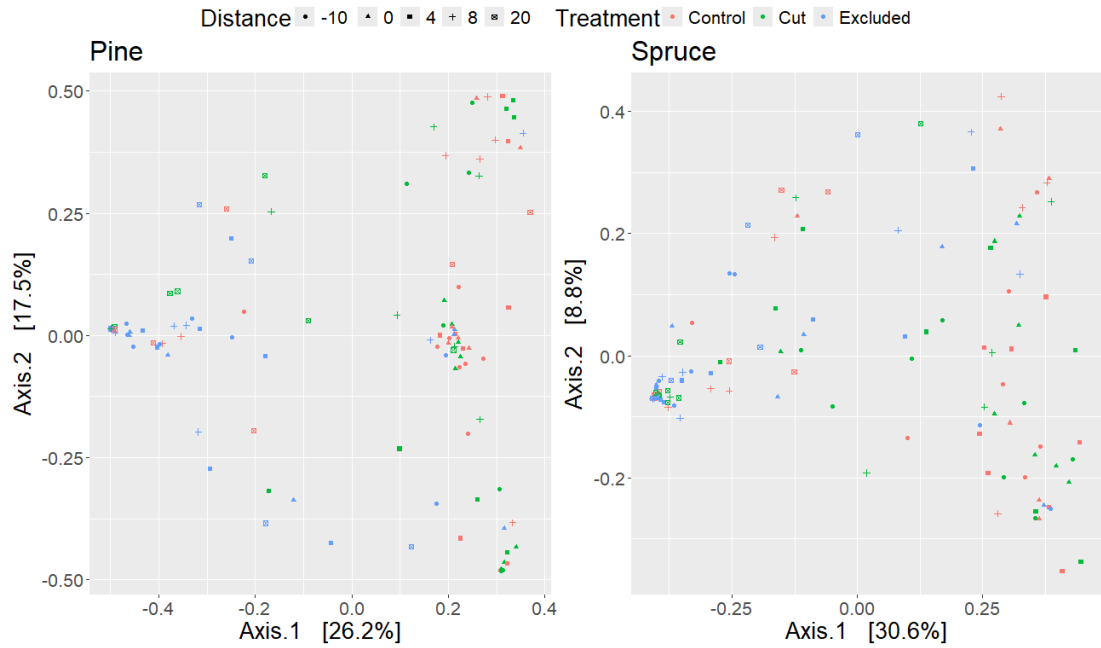


Figure 9 Principle Coordinate Analysis (PCoA) of ectomycorrhizal community composition in *P. sylvestris* and *P. abies*. Colours denote treatment (Control=red, Cut=green, Excluded=blue) and shapes denote distance from forest edge.

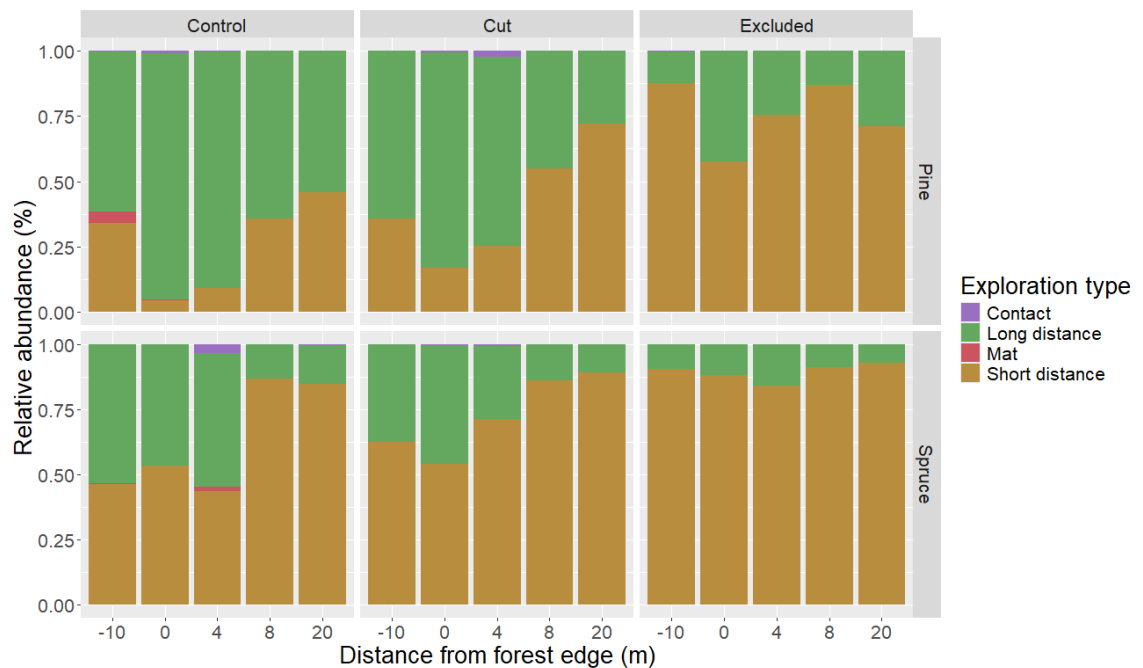


Figure 10 Relative abundance of ectomycorrhizal exploration types as defined by Agerer (2001) using the FungalTraits database. Exploration types have been further grouped into long distance, short distance with regards to life-history similarities. Long distance includes long-distance and medium-distance fringe. Short distance includes all short distance variants and medium-distance smooth. A clear pattern emerges where exclusion of competition from surrounding trees promotes establishment of short distance exploration types.

4. Discussion

This thesis aims to investigate whether competition in the edge zone of a harvested forest gap influences growth and N uptake of tree seedlings, as well as the EMF community composition on their roots. It combined a unique isotopic labelling approach combined with amplicon sequencing to investigate these edge-effects. Results from this study indicate a large effect of belowground competition on seedling productivity, through growth and N uptake. EMF community was also influenced by the edge effect and competition from surrounding trees on the host seedling.

In agreement with the first hypothesis, seedlings grew better when they were excluded from competition (Figure 2) by use of barriers in the soil. This effect was stronger than the distance-from-edge effect in cut and control seedlings where no significant positive trend was observed. This may be due to a delayed growth response in the seedlings. Previous studies on boreal tree species have shown no significant growth response of soil scarification, a way of removing competition, in the first year after planting (Löf 2000; Johansson et al. 2005), instead showing significant differences after two or three years (Örlander et al. 1996). This is likely due to seedling growth being greatly affected by the previous year's growing conditions.

Seedlings are able to use stored N from times of excess N supply, such as in a greenhouse before planting, for growth and maintenance at times of N limitation (Chapin 1980). The first year after planting, seedlings would be likely to grow well despite limited N uptake. This could limit the effect of competition and/or lack thereof during this time as they make use of stored N such as seen in this study. This would explain a decrease in N concentration from planting until sampling (Table 1, Figure 5) and could explain the non-significant differences in growth and N concentrations between the edge zone and clear-cut.

Contrary to hypothesis II, neither diversity nor richness of seedling EMF community significantly decreased with increasing distance from forest edge. This could be due to the planting of the clear-cut that occurred a few years before, meaning that the EMF community might have already re-established in the soil. This is supported by the fact that the exclusion treatment had a lower diversity and richness than the other two treatments, indicating that there may already be some competition with the established trees on the site. However, richness and Shannon's

diversity index are only broad indicators of community structure and do not show community composition.

Amplicon sequencing did reveal differences in abundance of long- and short-distance exploration types in line with hypothesis III (Figure 10). Removing competition appears to increase abundance of short-distance EMF genera, which is expected from previous studies. Sadly, due to time limitations it was not possible to do a statistical analysis of whether this had an effect on the N uptake to the seedlings. However, this change is likely driven by an increase in *Thelephora* sp. which is of a medium-distance smooth exploration type that has been grouped with short-distance exploration types due to their life-history similarities.

Thelephora sp. was the most abundant genera in the exclusion treatment for both pine and spruce. It's dominance in this zone is likely a combination of lack of competition along with priority effects. Previous sequencing work has shown that this is the most abundant genera found in boreal tree nurseries such as the one used in this study (Stenström et al. 2014). As it was likely already present at planting, intransitive competition may ensure competitive advantage for the already established species (Soliveres & Allan 2018). This priority effect has previously been observed in temperate Beech forests and also influenced subsequent community composition (Hiscox et al. 2015). Historic community composition determines current ecosystem diversity within many ecosystems (Kennedy 2010), and has been shown to occur within wood decomposing fungal communities which could also influence ecosystem functioning (Fukami et al. 2010). Whether the inclusion of *Thelephora* sp. from the plant nursery did influence the development of seedling EMF community composition is cannot be determined from this study, but it would be an interesting point for future research.

The priority effect was strongest in the excluded seedlings, whereas in the forest it was less abundant. Likely it is less competitive against the already established EMF such as *Suillus* sp. that is commonly found at these locales. *Suillus* sp. (long-distance exploration type) is host-specific to Pinaceae (Lofgren et al. 2018), which is in line with the results of this study (Figure 8). It likely has access to a greater supply of photosynthetically derived carbon from the larger trees it is connected to and use that to outcompete the less competitive *Thelephora* sp.

By cutting around the seedlings at the time of planting to sever all existing roots and mycelia growing in the planting zone, a growth increase compared to control of 8-85% was achieved, indicating a transient, positive effect on growth compared to control. This transitory effect is likely due to in-growth of mycelia into the cut area, exposing the seedling to competition again. It is difficult to say how long the competition-free period is, but studies using mesh bags filled with substrate that allow in-growth of mycelia have been commonly used in estimations of extramatrical ectomycorrhizal mycelia production since the early 2000's (Wallander et al. 2013). One such early study by Wallander et al. (2001) in south-

western Sweden using mesh-bags only allowing in-growth of mycelia, showed ample in-growth of EMF. By setting out the mesh-bags at different times of the year, they were able to show that mycelial growth was highest during autumn which would imply for this study that cut plots would be granted a relief period from external competition for a short period of time after planting. By autumn of 2022, the soil is likely to have been colonised by fast-growing EMF mycelia which by the following year would be followed by more slow-growing mycelia (Wallander et al. 2013) which would once again expose the seedlings to external competition.

Competition from already established EMF communities influenced the community composition on the seedlings. There is no record at this time of EMF community composition at this site before or after it was planted, however, given the heterogenous spatial distribution of EMF species, there will likely be some variation that cannot be explained by the treatments and distance-to-edge effects. Knowing this, the seedling community composition will likely vary by the spatial heterogeneity of the existing community composition. This was shown in the PCoA analysis of community composition, which showed a grouping of samples within treatments and distances (8 & 20 meters) where competition is expected to be low. Samples exposed to competition from surrounding trees did show higher variance with no clear pattern emerging.

5. Conclusion

With the changing public perception of clear-cuts, alternative harvesting methods such as CCF are likely to increase. This study bridges a gap between forest productivity and functional ecology of EMF, showing that belowground competition influences seedling growth. EMF community composition also changed on the seedlings with competition which could be influencing their ability to take up N. However, this study was not able to replicate the experiences from practical forestry showing a strong distance-to-edge effect on N-poor soils. A further time-perspective could be interesting, as EMF may also be varying in their ability to not only take up N but also store it over time in their extramatrical structures aiding in their competitive ability.

This study focused on exploration types of EMF as a proxy for their adaptation to soil N availability. This approach could provide a deeper look into the functional ecology of both seedlings and EMF with strong applications for productive forestry through *in-vivo* studies of different EMF community compositions and their response to fertilisation and soil preparation methods. By providing the right conditions for the seedlings that promotes N uptake through a specific EMF community may help mitigate the gap-edge effect. This study lays a foundation of understanding of the belowground factors that determine successful regeneration in northern boreal Sweden.

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Popular science summary

Regeneration has long been a central question in Swedish forestry. Especially in the northern boreal parts of Sweden where the ground close to large trees is devoid of any seedlings. A phenomenon that is called the gap-edge effect. To get around this problem, foresters have implemented clear-cutting, a method for harvesting trees where all trees are removed from an area. This limits the influence of the gap-edge effect as only the edge of the clear-cut area is in proximity to larger trees. While this has proven to be a reliable way to get an economic return from the timber the forest provides it also causes biodiversity losses and decreases the beauty of the landscape. As times have changed, the valuation of these last two values increased. Calls for a change in forestry practices, implementing different methods where the structure of the forests are kept largely intact after harvest are being proposed. However, implementing these clear-cut free forestry methods puts the problem of the gap-edge effect back on the map, leaving foresters scratching their heads on how to satisfy these demands while still being able to get an economic return from their forest.

This study shows that belowground competition is a big factor in the gap-edge effect. By being close to mature trees, seedlings are competing for nutrients such as nitrogen with the larger trees and the seedlings are at a disadvantage. The larger trees have a much more established network of ectomycorrhizal fungi that have taken up and keep a lot of the nitrogen from the little seedlings. These fungi act as an extended root network of the trees and there are many different species that come in many different shapes and sizes that are adapted to different conditions just like aboveground plants. As such, different species are found on the mature trees and on the seedlings. However seedlings experiencing the gap-edge effect have similar species to the mature trees. Whether this causes the gap-edge effect is still unknown, but it clearly shows that mature trees impact the growth of seedlings. As such, this study expands the understanding of how seedlings grow which with further research could unlock ways to implement new forestry methods in boreal Sweden.

Acknowledgements

I'd like to give huge thanks to everyone who's helped me on this thesis. First of all my supervisors Nils Henriksson and Andreas Schneider for their support and guidance throughout the process. Then I want to thank Lina Nilsson and Jasmin Danzberger for their help with labwork without which I'd still be standing by a lab bench. Of course I'd also like to thank the lab staff at the Department of Forest Ecology and Management for their assistance in making this thesis possible. Lastly, I'd like to thank Catherine Priemer for always believing in me and being a huge support every day!

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Ecosystem services from woody vegetation in East African rangelands
- 2023:17 Författare: Olivia Forssén
“It was a free and healthy job” – timber floating on the river Ångermanälven in the 20th century
- 2023:18 Författare: Kailey Tentis
Plant-soil feedbacks in boreal tree species
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- 2024:02 Författare: Rasmus Staaf
Riparian plant biodiversity and community composition of ditches and straightened streams. Identifying Key Environmental Drivers in a Boreal Forest Setting
- 2024:03 Författare: Noel Wieser
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