Forest edge effect on seedling growth, C/N allocation and fungal associations

A closer look at 3-year-old seedlings growth patterns above- and belowground and their mycorrhizal symbionts according to their distance to a forest edge

Julian Säflund

Master's Thesis project • 60 credits
Swedish University of Agricultural Sciences, SLU
Forest Ecology and Management
Jägmästarprogrammet
Examensarbeten / SLU, Institutionen för skogens ekologi och skötsel, 2025:03 • ISSN 1654-1898
Umeå, Sweden 2025

Forest edge effect on seedling growth, C/N allocation and fungal associations. A closer look at 3-year-old seedling growth patterns above- and belowground and their mycorrhizal symbionts according to their distance to a forest edge.

Julian Säflund

Supervisor:	**Nils Henriksson, SLU, Forest Ecology and Management**
Examiner:	Stefan Hupperts, SLU, Forest Ecology and Management

Credits:	60 credits
Level:	A2E
Course title:	Master thesis in Forest Science – Forest Ecology and Management
Course code:	EX0957
Programme/education:	Jägmästarprogrammet
Course coordinating dept:	Forest Ecology and Management
Place of publication:	Umeå, Sweden
Year of publication:	2025
Copyright:	All featured images are used with permission from the copyright owner.
Title of series:	Examensarbeten / SLU, Institutionen för skogens ekologi och skötsel
Part number:	2025:03
ISSN:	1654-1896
Keywords:	Edge effect, seedling, competition, growth, N, ectomycorrhiza, Boreal, forest regeneration

Swedish University of Agricultural Sciences
Faculty of Forestry
Department of Forest Ecology and Management

Abstract

Boreal forest growth is largely limited by nitrogen (N) availability, leading to intense belowground competition among trees. Most boreal tree species form ectomycorrhizal (ECM) symbioses, where fungi enhance nutrient and water uptake in exchange for carbon. Following clearcutting, early-stage ECM fungi—adapted to colonize roots quickly and access mineral N—tend to dominate. As stands mature and nutrients become scarcer, later-stage ECMs capable of acquiring complex organic N forms become more competitive and prevalent.

This thesis examines the growth, nitrogen allocation, and ECM fungal community composition of 3-year-old pine (*Pinus sylvestris*) and spruce (*Picea abies*) seedlings along a distance gradient from a mature forest edge into a 7-year-old clearcut in northern Sweden.

Seedlings increased in height, dry weight, and N uptake with distance from the forest edge, with a distinct growth threshold observed between 4 and 8 meters.

Seedlings surrounded by a root exclusion barrier were generally larger and unaffected by distance, suggesting that belowground competition for nitrogen strongly limits early seedling growth. Simultaneously, the ECM fungal community composition shifted along the same gradient: early-stage ECMs were more abundant on the clearcut, while late-stage ECMs were more abundant closer to or within the forest.

These findings underscore the influence of spatial gradients and root competition on both seedling performance and ECM community structure, with implications for forest regeneration and the management of edge effects in boreal silviculture.

Keywords: Edge effect, seedling, competition, growth, N, ectomycorrhiza, Boreal, forest regeneration

Table of contents

List of figures

Abbreviations

Abbreviation	Description
N	Nitrogen
C	Carbon
DW	Dry weight
ECM	Ectomycorrhiza
OTU	Operational taxonomic unit
ITS2	Internal transcribed spacer 2

1. Introduction

1.1.1 Belowground competition in the Boreal and regeneration troubles

Boreal forest growth is limited by tree nitrogen (N) acquisition which sets up a fierce competition among trees of same and different species. Swedish forestry is dominated by rotational management, with clearcut harvests, followed by regeneration.

Although there are several negative effects from clearcutting such as decreasing biodiversity or lower esthetic values for the public, it is often said to be the only effective way of regenerating a forest stand in N-limited regions. Scots pine, *Pinus sylvestris*, covers 37 % of Sweden and is a known pioneer species and needs a relatively open area with little overshadowing competition in order to regenerate (Länstyrelsen n.d.). Other species such as Norway spruce, *Picea Abies*, can slowly establish beneath a full forest cover. Therefore the various forms of continuous cover forestry often hamper efficient pine seedling regeneration, thus hindering efficient wood production (Jakobsson 2005; Axelsson et al. 2014).

With clearcuts, seedlings get a fresh start without any competition from older trees with well-established roots. Except at the forest edge where the older forest stand has its roots spread into the clearcut competing with the seedlings. The edge effect on wood production is surprisingly strong, with up to 90 % volume growth decrease in the first 5 m (Jakobsson 2005). With current political movements, the EU and the public are pushing to limit clearcut forestry and for reducing the size of clearcut patches meaning more edge affected area per clearcut (*New EU Forest Strategy for 2030* 2021; *The European Green Deal - European Commission* 2024). For example, a common clearcut in Sweden is ca 10 ha, if the stand is a perfect square with an assumed 5 m edge effect then it will have ca 6,4 % edge affected area. If clearcuts are now only allowed to be 1 ha, like in certain parts of Germany (*Forest Act for Baden-Württemberg* 1996), it will have ca 19 % edge affected area. With uneven shaped stand, the edge affected area increases even more. In addition to the decreased volume growth, seedling regeneration (height and diameter) is also hampered which only worsens the situation (Axelsson et al. 2014; Häggström et al. 2024). Improved scientific understanding of such edge effects is crucial in order to avoid production losses if clearcuts are to become smaller.

1.1.2 Biomass partitioning and nitrogen allocation depending on resource availability

Seedlings, young and even mature trees have long been observed to allocate their growth differently depending on the nutrients available to them. Trees in N-fertilized stands have an increased growth in their aboveground structure (Linder & Troeng 1980; Tamm 1991; Nohrstedt 2001), including stem growth and needle biomass (Axelsson & Axelsson 1986; Nohrstedt 2001). These trees produce an increased number of needles which are also bigger with a higher N concentration (Linder & Troeng 1980; Axelsson & Axelsson 1986). With an addition of 150 kg N/ha, an extra stem growth up to $15m^3$/ha can be observed over a 10-year period (Nohrstedt 2001). A long-term study showed that 20-30 years after N-fertilization on the mature trees from the first generation, the height growth increased in the 10-year-old spruce trees in the second generation (From 2014). Belowground, however, the relative growth of fine roots decreases. Axelsson & Axelsson 1986 reported that the relative distribution of annual dry matter production of fine roots decreases from ca 30 % to 15 % with N addition.

Although not studied in this thesis, seedlings also appear to allocate growth differently depending on which chemical form of N is available. Organic N promotes root growth more than inorganic N (Cambui et al. 2011; Gruffman et al. 2012).

1.1.3 Mycorrhizal association and succession

Nearly all plants in the Boreal have some kind of mycorrhizal symbiosis. Trees are no exception. In pine and spruce stands ectomycorrhizae (ECM) colonize up to 100 % of all tree root tips (Martin et al. 2001; Mahmood et al. 2002). Ectomycorrhizal symbionts provide their tree host with increased nutrient (Smith & Read 2008; Becquer et al. 2019) and water uptake (Lehto & Zwiazek 2011; Becquer et al. 2019) in exchange for carbon.

This partnership is sometimes questioned, during severe N-limitation the symbiont can instead act as a parasite by incorporating increasing proportions of absorbed N into its own fungal biomass, while still getting C from its host (Näsholm et al. 2013). Newer studies have even found that trees connected to the same symbiont disfavor each other by competing for the same nutrient pool (Henriksson et al. 2021a). According to the surplus-C hypothesis, that bigger trees act as a bigger C-source and/or N-sink (Bunn et al. 2024), small seedlings connected with mature trees via the same symbiont should be more disfavored than if connected with other equally small seedlings. Meaning a seedling's nutrient and water uptake should be more challenging when surrounded by mature trees.

Another key component in plant N nutrition, especially in boreal forest soils, is that N naturally occurs in multiple chemical forms attracting different kinds of fungi. These N forms can be generally divided into mineral (ammonium and nitrate) and organic forms (amino acids, peptides, proteins, and other partially decomposed organic material). After a clearcut, most of the symbionts die along with their host leaving a free path for saprotrophs to feast and proliferate on all the fresh litter left by the felling (Kyaschenko et al. 2017). As the organic matter is decomposed by the saprotrophs, nutrients are released and as the seedlings grow, mycorrhizal symbionts start to recolonize the soil. These early-stage ECMs specialized in either colonizing or absorbing easily accessible mineral N typically dominate on clearcuts (Lilleskov et al. 2011; Kyaschenko et al. 2017). As time goes and nutrients get sparser, later-stage ECMs with the capacity to absorb and decompose more complex N forms become more competitive and thus dominant in more mature stands (Bödeker et al. 2014; Kyaschenko et al. 2017; Jörgensen et al. 2025).

1.2 Aim of the thesis

This thesis investigates pine and spruce seedling's growth, N allocation and mycorrhizal community composition on seedling roots, as functions of the seedlings' distance to a forest edge. Using different treatments to exclude belowground competition completely or temporarily, combined with a unique stable isotope labelling technique, 3-year-old seedlings' growth above- and belowground will be studied along with their nitrogen uptake and allocation. Fungal ITS2 amplicon sequencing of the seedling fine roots provides data on the community composition of mycorrhizal symbionts occupying their root system.

1.3 Hypotheses

(i) Isolation from belowground connection to surrounding roots and fungal mycelia leads to enhanced seedling growth and N uptake. This effect should be strongest near mature trees, where potential competition is greater.

(ii) Seedlings growing in the forest/near mature trees have a higher root/shoot ratio and are smaller than seedlings growing in the open clearcut.

(iii) The ectomycorrhizal community composition differs between forest and clearcut, with species associated with later successional stages being present in the forest but absent in the clearcut.

(iv) Root isolation does not affect ectomycorrhizal species composition.

(v) Bigger seedlings have greater ectomycorrhizal species diversity.

2. Materials and methods

2.1 Experiment site, layout and treatments

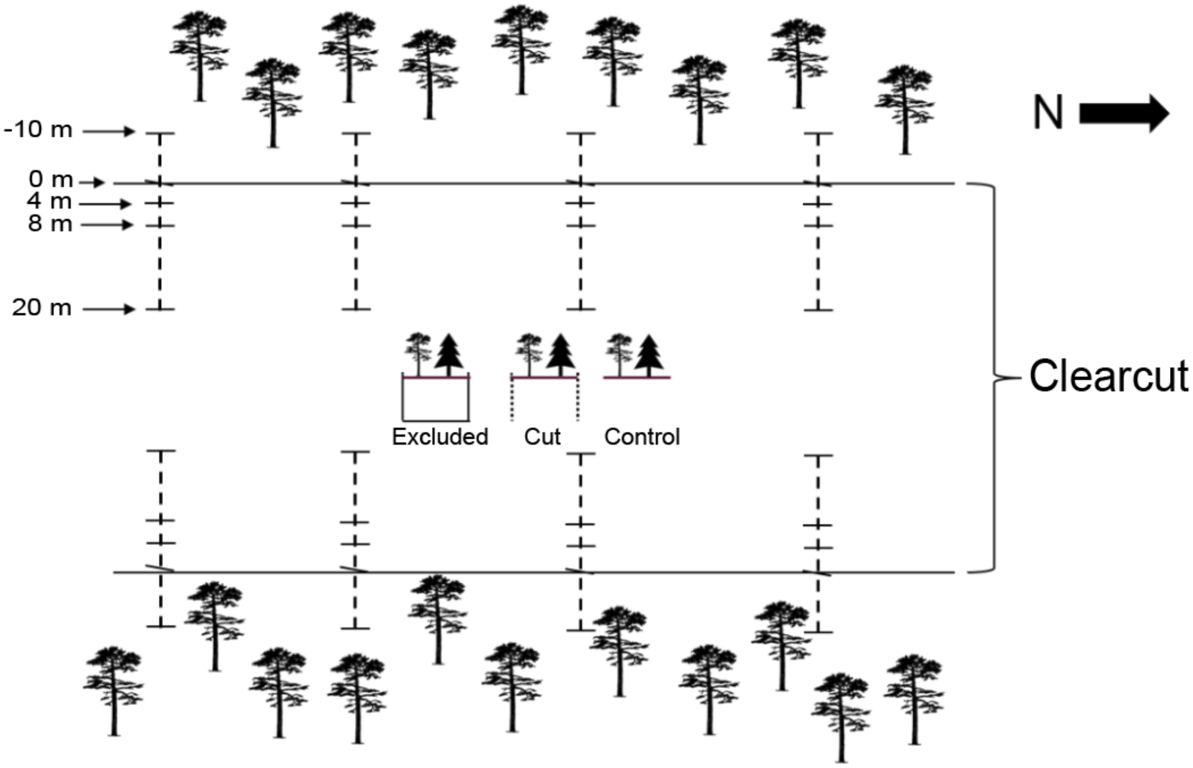


Figure 1. Illustration of the site at Torrböle, northern Sweden (Björs 2024:11).

The experiment site is located outside of Torrböle, ca 35 km southwest of Umeå, Västerbotten in northern Sweden. It is a dry sandy site with lingonberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*). At the site there are eight transects (labeled A to H) with five plots each placed at -10 m, 0 m, 4 m, 8 m, and 20 m from the forest edge (ca 70-year-old pine) and into a 7-year-old clearcut which has been mounded and planted with pine seedlings (fig. 1). Each plot has five subplots (labeled 1 to 25) where the experiment's seedling pairs (pine and spruce) were planted in June 2022. Before being transferred to the site, the seedlings were isotopically enriched to 1 atom% ^{15}N. Each subplot was composed of three treatments and five seedling pairs. One pair was planted into a bucket filled with the native field soil, this was the root isolation treatment (named root excluded) which acts as an impermeable barrier for all roots and fungal hyphae from the surrounding soil. The bottom of the buckets were fitted with 45 µm nylon mesh, for water drainage. For the second treatment (named cut), one pair was planted into the soil and had its surrounding soil severed with a spade to kill any roots. This was to create an initial condition free of belowground competition, although neighboring roots could eventually regrow and resume competing with the seedlings. The final treatment was a control, where the seedlings were simply planted into the soil without any additional disturbance.

2.2 Work flow

During September of 2024, a seedling pair with each treatment from one of the subplots were carefully dug up to keep the roots as intact as possible. After cleaning the roots from dirt and debris, they were put on dry ice to preserve the DNA of any mycorrhizae. A total of 238 seedlings were dug up, but only 180 seedling roots were put on dry ice. The first two transects were not put on ice because of a change in the time plan.

Fungal DNA was extracted from a subsample of each root system, and the remaining part was put in an oven along with the stems to dry (60 °C for 2-3 days).

The root subsamples were ground to a powder with the help of liquid nitrogen, and DNA extraction was done using a QIAGEN DNeasy Power Soil kit and DNeasy Plant Pro kit. The DNA samples where then sent to Novogene in Munich, Germany for Illumina ITS2 region sequencing. During the extraction, I noticed the DNA concentration was low and so a small metal bearing was added during the first step where the cell membranes are crushed.

The dry stem, needles and roots samples were weighed separately, and the needles and roots were crushed/mixed to a powder using a tube mill (IKA PowerMill). Next, 5 mg of the needle and root sample was sent for isotopic analysis of ^{15}N and ^{13}C at the SLU Stable Isotope Laboratory, SSIL, combining isotope ratio mass spectrometry (DeltaV, Thermo Fisher Scientific, Bremen, Germany) and an elemental analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany). See Werner et al. 1999 for a more detailed explanation. Natural ^{15}N abundance is represented by reference samples taken from six different spruce and pine saplings at the experimental site.

2.3 Statistical analyses and data handling

The biomass and nitrogen data were first organized in excel and analyzed using JMP Pro 17 and 18. Models are standard least squared models with full factorial and are explained in supplementary information. Normality was tested using Shapiro-Wilk test. In cases of unevenly distributed residuals, the data was log-transformed before analysis.

DNA data was put through a pipeline and further analyzed in R, see 2.3.5 for further information.

The letters on the graphs indicate significance, where groups with different letters are significantly different from each other ($\alpha = 0,05$) and groups with letters in common are considered statistically similar.

2.3.1 Isotopic values

Isotopic values, or fractions, are helpful indicators when it comes to deciphering growth allocation and uptake. The data from SSIL contains mass fractions of N and C, delta ^{15}N and ^{13}C of which atom% ^{15}N and ^{13}C are derived.

The mass fraction is g N or C per g of dry mass sample, so a concentration of each element. Delta (δ) ^{15}N and ^{13}C are expressed as permille (‰) difference from a universal standard reference point. This standard is different for each element and is a ratio between two isotopes, in this case ^{15}N/^{14}N and ^{13}C/^{12}C. A delta of 0 means the sample has the same ratio as the standard. A delta of 100 means the sample is 100 ‰ different from the standard, or 10 % different. A higher delta means there is relatively more ^{15}N or ^{13}C compared to the standard and vice versa for a lower delta. Due to the way delta is calculated, it grows in error with high values (above 100 ‰ and below -100 ‰), which is why atom% is preferred in these cases.

Atom% is the percentage of the isotope ^{15}N out of all the N (or ^{13}C to all C). In this thesis, atom% will be used since my samples are heavily enriched with ^{15}N ($\delta\,^{15}N_{max}$ = 1888 ‰).

A more detailed explanation can be found in *Stable Isotope Ecology* by Brian Fry (2006).

2.3.2 Damaged/odd samples

In total, 24 seedlings were damaged, either from grazing or other natural causes. A t-test was conducted to see whether these seedlings significantly differ from the un-damaged seedlings regarding all variables: weight of needles, stem and root, and height growth for each year. For all variables except one they did not, and so were kept in the dataset for further analyses. Only for the growth of the year 2022 was the difference significant. While analyzing with the growth in 2022, the damaged seedlings were removed from the dataset. Keeping the damaged seedlings in the remainder of analyses, of course, decreases the true weight but the change is insignificant and deemed reasonable.

Corrections for soil contaminated root samples and isotopic measurement errors are explained in the supplementary information.

2.3.3 DNA handling and fungi classification

Raw DNA sequences from Novogene were put through a pipeline called Pipits, which merges forward and backward reads, removes bad-quality reads, clusters sequences with 97% similarity into OTUs (Operational Taxonomic Units), and identifies known OTUs with the UNITE database. For a more detailed explanation of the pipeline and the UNITE database, visit https://github.com/hsgweon/pipits and https://unite.ut.ee/. The DNA data was later processed in R-studio (v4.2.2), where it was standardized via rarefaction (a form of bootstrapping) down to the lowest sequencing depth of 17 266 reads. Information about fungal lifestyle, morphology and exploration type was retrieved from FungalTraits by Põlme et al. (2021) and added to the corresponding OTU.

Non-metric Multi-Dimensional Scaling (NMDS) were made using ordination with Bray distance (package: phyloseq). PERMANOVA models for ectomycorrhizal community composition are in function of distance, treatment, host species and their interactions with 9999 permutations (package: vegan. function: adonis2) and pairwise comparison were done with a Bonferroni-adjusted PERMANOVA with 9999 permutations (package and function: pairwise.adonis). Since the PERMANOVAs are done using permutations, no p-values will be given since they slightly change each time the model is run. Ectomycorrhizal community diversity parameters were checked for normality with a Shapiro-Wilk test and later compared with either Tukey's or Wilcoxon rank-sum test.

Classification of ECM genera into either early- or late-stage was done with the help of literature, and not from my results. Although I did class my most abundant ECM OTUs. I classified *Suillus, Lactarius, Laccaria* and *Tylospora* as early-stage and *Tricholoma* and

Piloderma as late-stage (Lilleskov et al. 2011; Clemmensen et al. 2015; Kyaschenko et al. 2017; van der Linde et al. 2018; Jörgensen et al. 2023, 2025). Individual genera of early- and late-stage were tested with Kruskal-Wallis and compared with Benjamini-Hochberg adjusted Tukey's test, while the groups early- and late-stage were tested with an ANOVA and compared with Tukey's test.

Upon data inspection, 10 outliers were removed. Unfortunately, five of the outliers were control samples at -10 m (three spruce and two pine seedlings). See supplementary information fig. S5-S8 for further details.

3. Results

3.1 Seedling overview

Root exclusion tended to increase seedling total biomass (dry weight) compared to controls and seedlings in the cut treatment. The mass distribution was also affected, with control and cut seedlings being more skewed toward the smallest biomass (75-95 % seedlings below 5 g). The root excluded seedlings had a more even biomass distribution and only 8-23 % weighed below 5 g (fig. 2).

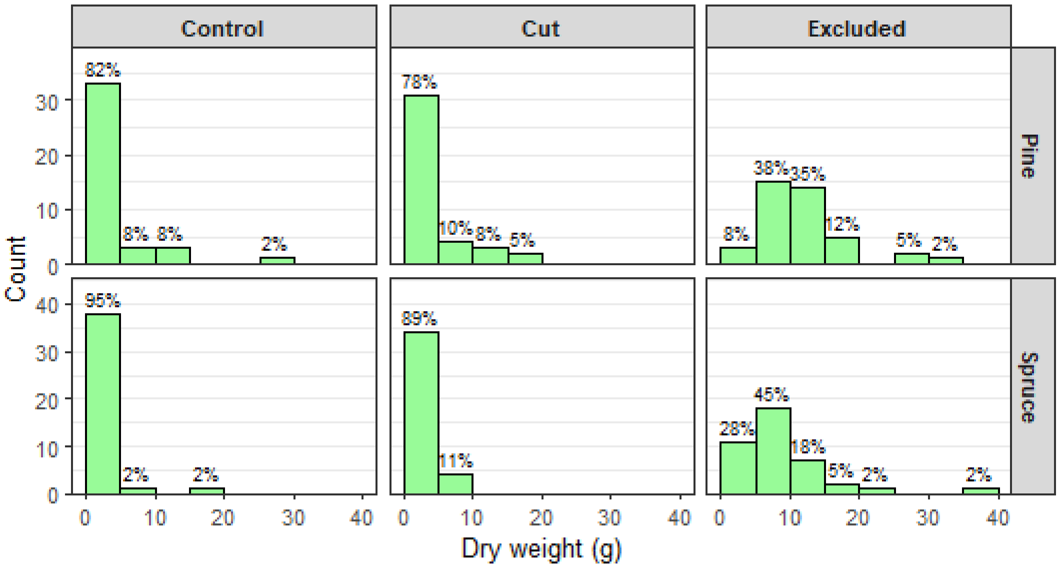


Figure 2. Distribution of the seedlings' total dry weight. Control and cut seedlings were skewed towards smaller sizes, while root excluded seedlings were bigger and more evenly distributed.

Seedlings growing at least 8 meters from the forest edge were taller than those growing in, or near, the forest. A height growth threshold could be seen between 4 and 8 m from the forest edge. The threshold was not detectible after one season of growth, but the accumulated growth of pines and spruces after two and three growing seasons was greater beyond the 4 m mark for both control and cut. Besides seedlings being taller, the growth per year also increased faster beyond the 4 m mark. Height growth of root excluded seedlings did not follow the same pattern and were unaffected by their proximity to the forest edge (fig. 3).

After three years, seedlings in the cut treatment were significantly taller than control ($p = 0,0118$). During the second growth year (2023), cut seedlings' annual growth was also significantly higher than control ($p = 0,0017$). Excluded seedlings were significantly taller after three years ($p < 0,0001$), and their annual growth was also significantly higher than control and cut during the second and third year ($p \leq 0,0033$) (fig. 3).

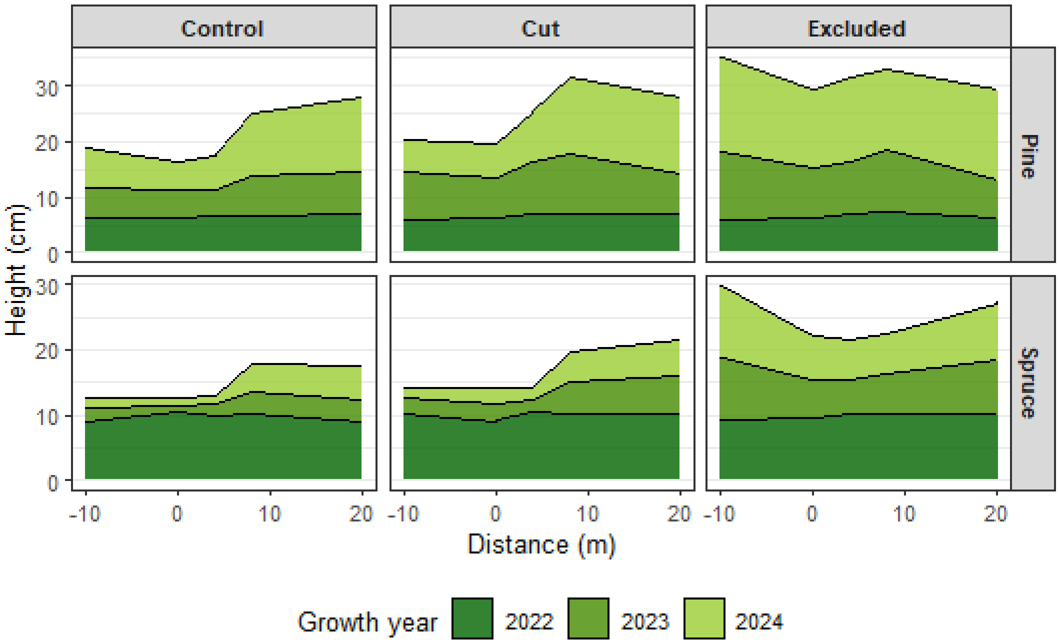


Figure 3. Height of seedlings for each year. Both control and cut seedlings were taller beyond 4 m. Root excluded seedlings were generally taller and unaffected by their distance to the forest edge. Damaged seedlings have been removed.

3.2 Biomass and Nitrogen

3.2.1 Dry weight and total N

Upon data inspection, one outlier (spruce seedling at 4 m with the cut treatment) was removed due to probable weighting error. This affects the dry weight, biomass allocation and total N content/mass.

Seedlings growing in, or near, the forest had 42,4 % smaller biomass (root + shoot), and lower total N content (52,1 % less in needles and 32,9 % in roots), compared to 8 m and beyond (fig. 4a, $p \leq 0{,}0001$). After three growing seasons, root excluded seedlings were the largest at all distances (10,4 ± 6,6 g), control treatments were the smallest (2,9 ± 4 g), and cutting the surrounding soil produced intermediate biomass (3,5 ± 3,4 g) (fig. 4b, $p < 0{,}0001$). Root excluded seedlings also had significantly more N mass (126,5 % more in needles and 348,2 % in roots) than control and cut seedlings (fig. 4b, $p \leq 0{,}0001$). Both control and cut seedlings grew larger with increasing distance from the forest edge and accumulated more N, whereas this distance did not significantly affect the root excluded seedlings (fig. 4c, S9, $p \leq 0{,}0106$).

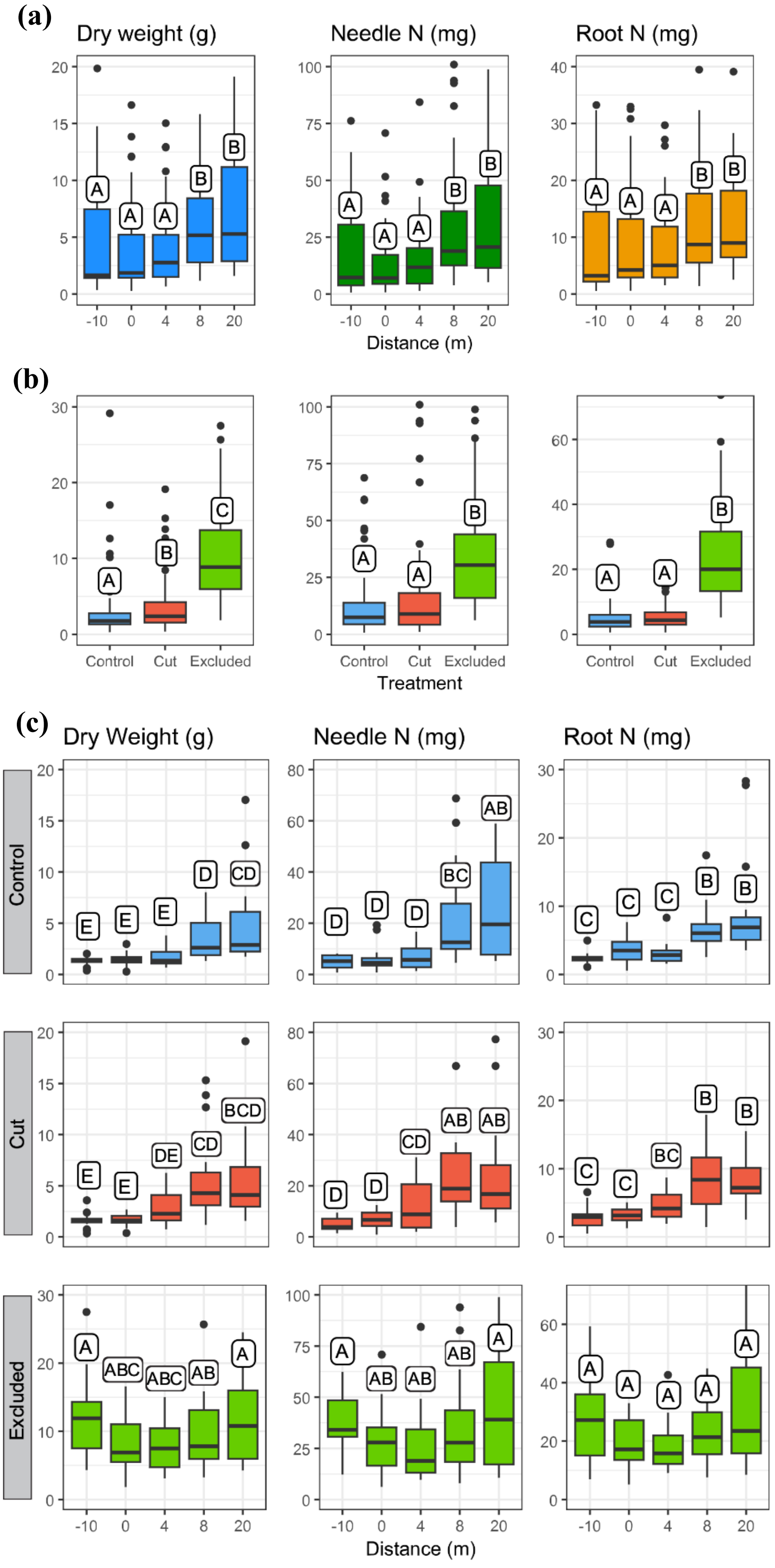


Figure 4. Total dry weight (g), and needle and root N content (mg) in relation to the seedlings' distance to the forest edge (a), their treatment (b), and their interaction (c). The significance letters are figure and dataset dependent. Splitting the data into species did not alter the trends or significances. In general, weight and N content were higher beyond 4 m, excluded seedlings exhibited higher values but were unaffected by their proximity to the forest. OBS: different scales on the y-axes.

The effect of releasing seedlings from belowground competition was quantified by normalizing the dry weight of root excluded seedlings by the corresponding control seedlings at each distance from the forest edge. The benefit of release from competition was significantly greater for seedlings growing in or near the forest, compared to seedlings growing at least 8 meters into the clearcut (fig. 5). Root exclusion in the forest was significantly more beneficial than on the clearcut, for both the dry weight and the N content of the seedlings ($p \leq 0{,}0001$). The seedlings dry weight increased by 8,2 in the forest and 3,2 on the clearcut. When modelling the N content split by tissue type (needle and root), only the needles' N content was significantly more benefited by root exclusion in the forest compared to the clearcut, increasing by 8,5 instead of 2,5 ($p = 0{,}0036$). The nitrogen content of roots increased by 10,6 in the forest and by 7,2 on the clearcut, but this difference was not significant. There was no significant difference between spruce and pine for either DW or N content ($p \geq 0{,}3118$).

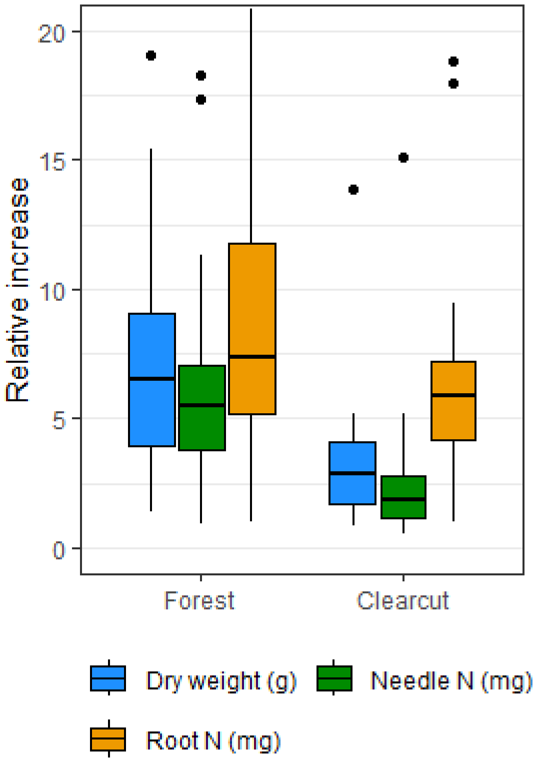


Figure 5. Excluded seedlings' relative increase in total dry weight (g) and N content (g) in needle and root compared to control. Root exclusion in and near the forest is more beneficial than on the clearcut.

3.2.2 Biomass allocation

Spruce seedlings generally had a higher root/shoot ratio, around 0,86 while pine seedlings had 0,58 ($p < 0{,}0001$). The spruce seedlings ratio remained unaffected by treatment whilst for pine seedlings, root exclusion led to significantly more relative root mass (59 %) than for control and cut treatment (fig. 6a, $p = 0{,}0005$). However, analyzing tree species separately, I found that excluded spruce seedlings had more root mass (22,3 %) compared to cut spruce (fig. 6a, p = 0,0255). Overall, distance had no effect on seedling root/shoot ratio (p ≥ 0,1445), except for control pine seedlings which had more root mass on the forest edge than furthest away (fig. 6b, p = 0,0202).

With distance as a continuous variable, DW of needles, stem and root all significantly increased with distance for control and cut seedlings (p ≤ 0,0121) but remained unaffected in excluded seedlings (p ≥ 0,1265). Root/shoot ratio was also stable across distance, with the one exception of pine seedlings from the cut treatment which showed higher root/shoot ratio in the forest, decreasing with distance (fig. 6c, p = 0,0369).

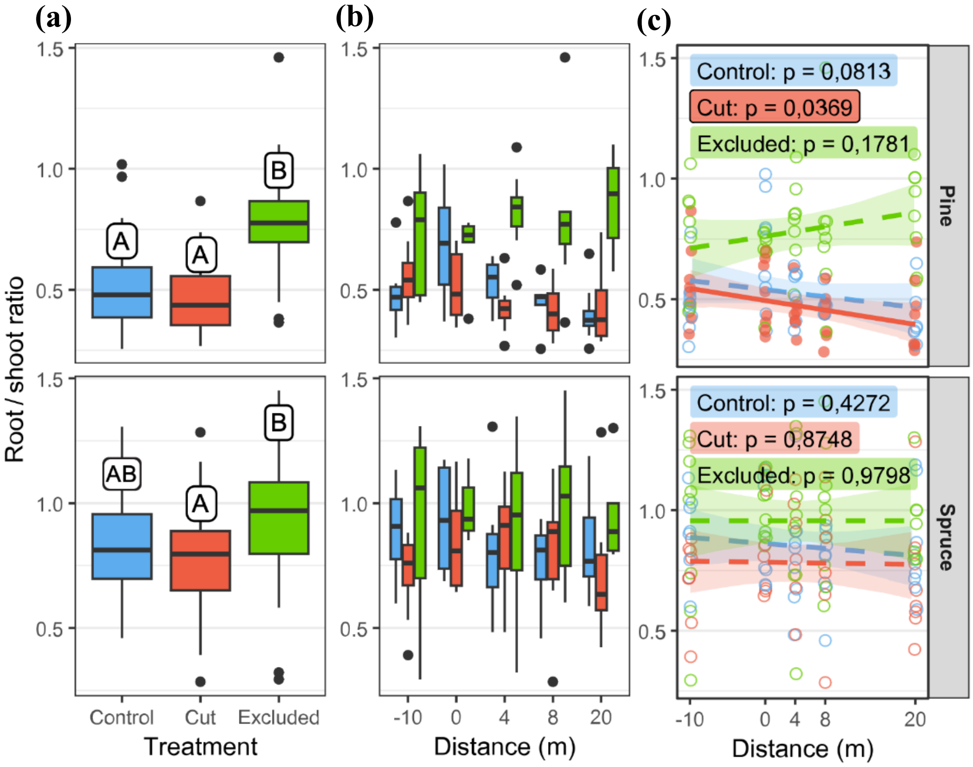


Figure 6. Root/shoot ratio per treatment (a), per distance as a factor (b) and as a continuous variable (c). Cut pine and spruce seedlings and control pine seedlings have significantly less root mass than excluded (a). Control pine seedlings had more root mass on the forest edge than furthest away (b). Only pine seedlings in the cut treatment significantly decreased their relative root mass (c). Dashed lines with hollow dots are not significant (p > 0,05), while whole lines with filled dots are significant (p ≤ 0,05).

3.2.3 Atom% ^{15}N

Control and cut seedlings atom% ^{15}N in the roots and needles were significantly more and more diluted with distance (p ≤ 0,0033). This indicates that N uptake was higher further out on the clearcut than in the forest. The isotopic dilution in excluded seedlings was not affected by distance (fig. 7, p ≥ 0,3932).

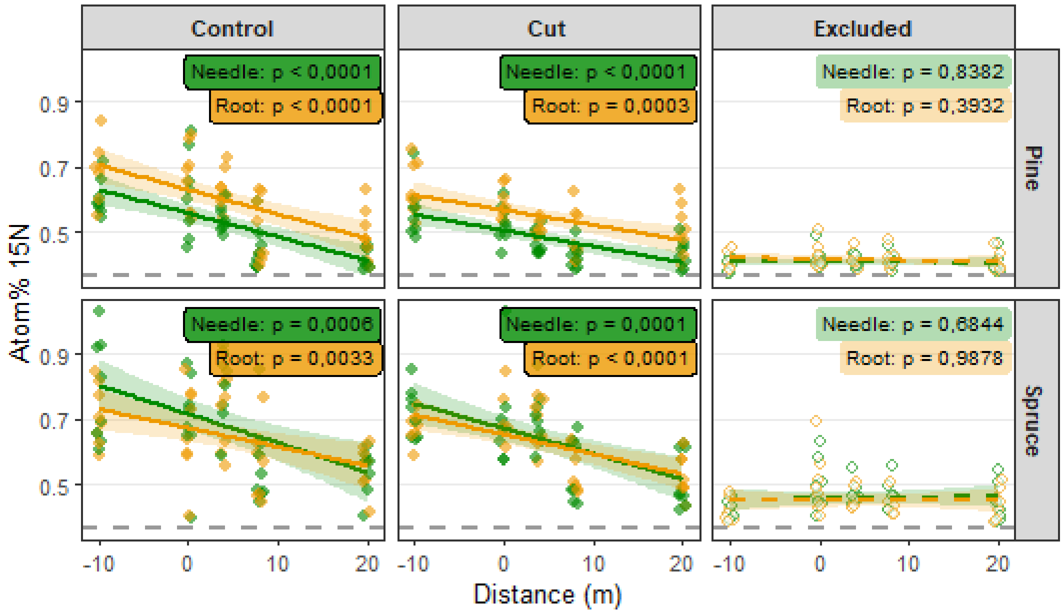


Figure 7. Atom% ^{15}N dilution in the seedlings' needles and roots. Distance significantly increased control and cut seedlings' ^{15}N dilution. Excluded seedlings were unaffected by distance and were equally diluted. The dashed grey line at the bottom is the natural abundance for each species (0,3653 for pine and 0,3651 for spruce). Dashed lines with hollow dots are not significant (p > 0,05), while whole lines with filled dots are significant (p ≤ 0,05).

3.2.4 N-concentration

N-concentration of control and cut pine seedlings needle significantly increased further away from the forest (p ≤ 0,0182). All pine roots and root excluded pine seedlings' N-concentration were unaffected by their proximity to the forest (p ≥ 0,3326).

For spruce, needle N-concentration of cut seedlings increased with distance, and roots of excluded seedlings decreased. Control spruce was not affected by distance to the forest edge (fig. 8, p ≤ 0,0417).

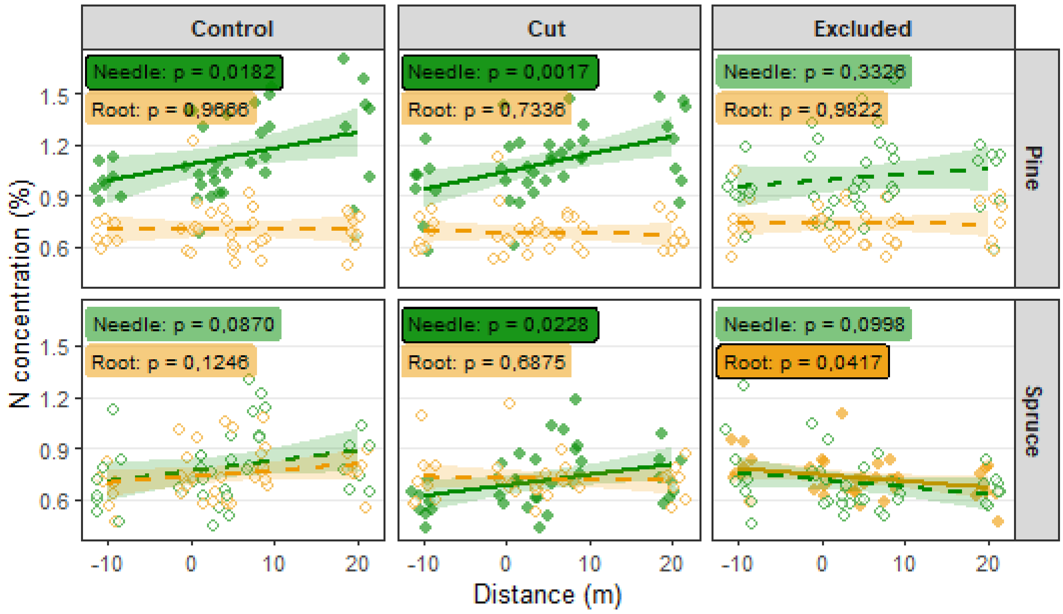


Figure 8. Seedlings' needle and root N-concentration. The N-concentrations significantly increased in the needles for control and cut pine seedlings. For spruce, the needles of cut seedlings significantly increased their N-concentration and for excluded seedlings, their roots significantly decreased their N-concentration. Dashed lines with hollow dots are not significant (p > 0,05), while whole lines with filled dots are significant (p ≤ 0,05).

3.3 Fungal symbionts

3.3.1 Kingdoms and ECM primary lifestyle

At the end of the quality controls 141 samples remained. 13 457 OTUs were found out of which 61,4 % or 8 269 OTUs were fungi but made up 91 % of the total reads. Moving forward, abundances (relative, mean, or absolute) will be based on only fungal OTU reads.

The non-metric multidimensional scaling (NMDS) of all fungal OTUs showed no clear separation between distances or treatments, but pine seedlings were more grouped than spruce seedlings. There is a spread across both axes indicating some hidden variable/s (fig. 9). Ectomycorrhiza and saprotrophs each make up on average ca 25 % of the relative abundance of total reads. Unknown OTUs account for 38,9 % and the remaining 9,8 % is composed of various pathogens, parasites, lichens or other irrelevant symbionts such as arbuscular mycorrhiza (fig. 10). When zooming in and observing only ECM OTUs, the NMDS lost the host species grouping (fig. S12).

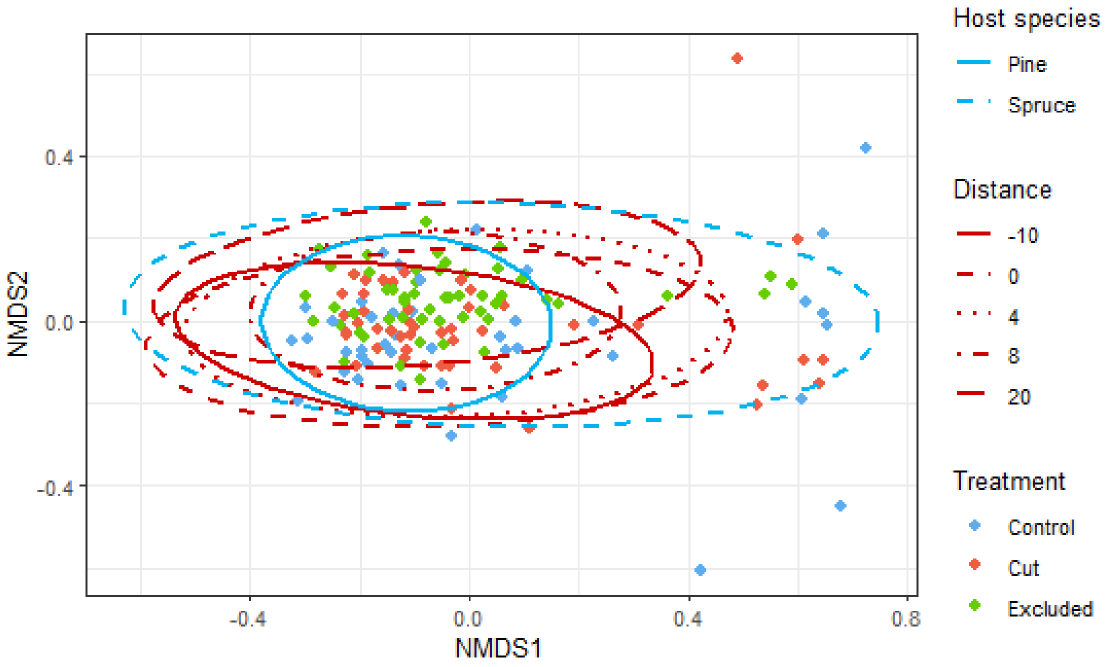


Figure 9. NMDS of only fungal OTUs colored by treatment and circled by distances to the forest edge (red) and host species (blue). No clear grouping can be seen between treatments or distances, indicating similar fungal communities. The fungal community associated with pine seedlings seem to be more similar than on spruce. There is a spread along the NMDS1 axis indicating some hidden variable/s.

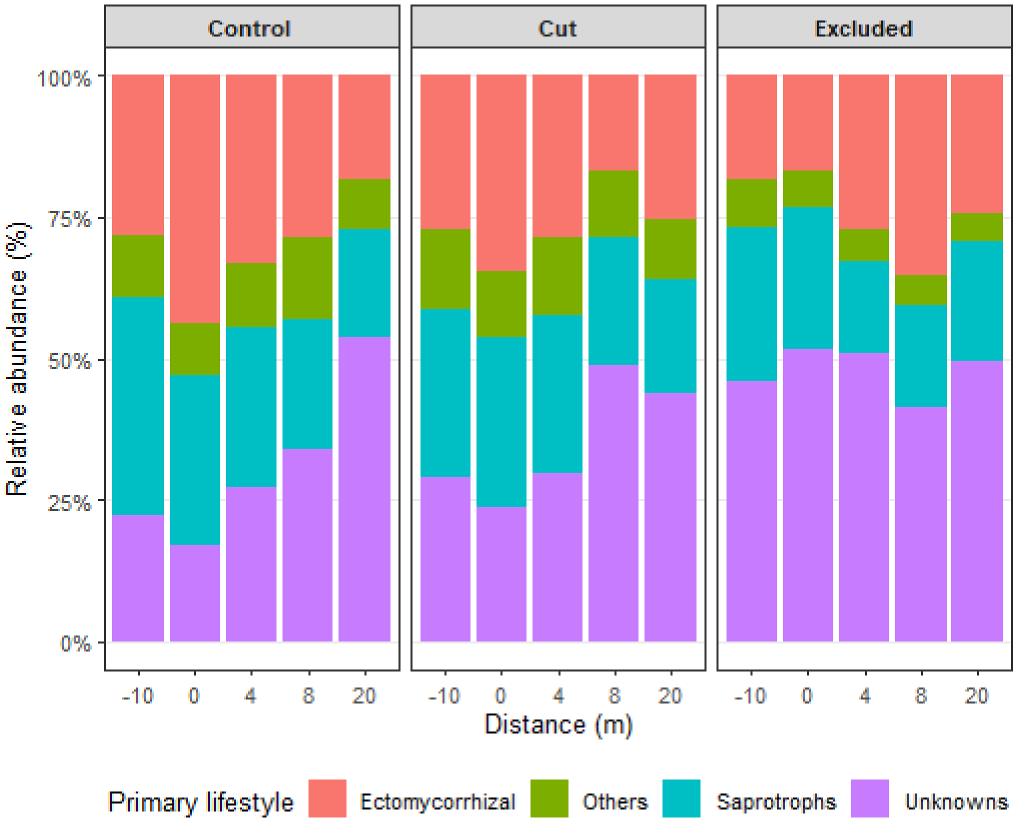


Figure 10. Relative abundance of fungal primary lifestyles. ECMs and saprotrophs each make up ca 25 % of the reads, unknows 38,9 % and others (ex. parasites, lichens, or other types of mycorrhizae) 9,8 %.

3.3.2 ECM community composition and diversity

Community composition

As distance from the forest edge increased, the ECM community composition gradually changed. The most notable visual change is that the relative abundance of *Suillus* increased with distance, while *Lactarius* and *Piloderma* decreased. Late-stage ECMs (*Piloderma* and *Tricholoma*) had a higher mean abundance in, or near, the forest while early-stage ECMs (*Suillus*, *Lactarius*, *Laccaria* and *Tylospora*) had a higher mean abundance further out on the clearcut (fig. 11).

Root excluded seedlings ECM composition significantly differed from control. The 20 most common OTUs are shared among the three treatments, but in different abundances. *Lactarius* and *Tomentellopsis* are relatively more abundant on the excluded seedlings, while *Elaphomyces*, *Piloderma* and *Tricholoma* are more common on control. Late-stage ECMs had a significantly ($p < 0,0001$) higher mean abundance on control and cut seedlings compared to root excluded (fig. 12).

ECM community composition also significantly differed between pine and spruce seedlings. The most notable difference is that *Suillus* is common on pine and *Tylospora* is common on spruce (fig. 13). Pine had 146 unique OTUs while spruce only had 51 unique OTUs. Together, pine and spruce seedlings shared 164 ECM OTUs.

When modeling with only the dry weight of the host seedling, it had a significant effect on ECM composition. However, DW correlated with distance (Pearson ca 0,44 and Spearman ca 0,64) which had the highest Sum of Squares in the previous model. For example, *Suillus* increased with DW but we know from previous results that DW increases with distance.

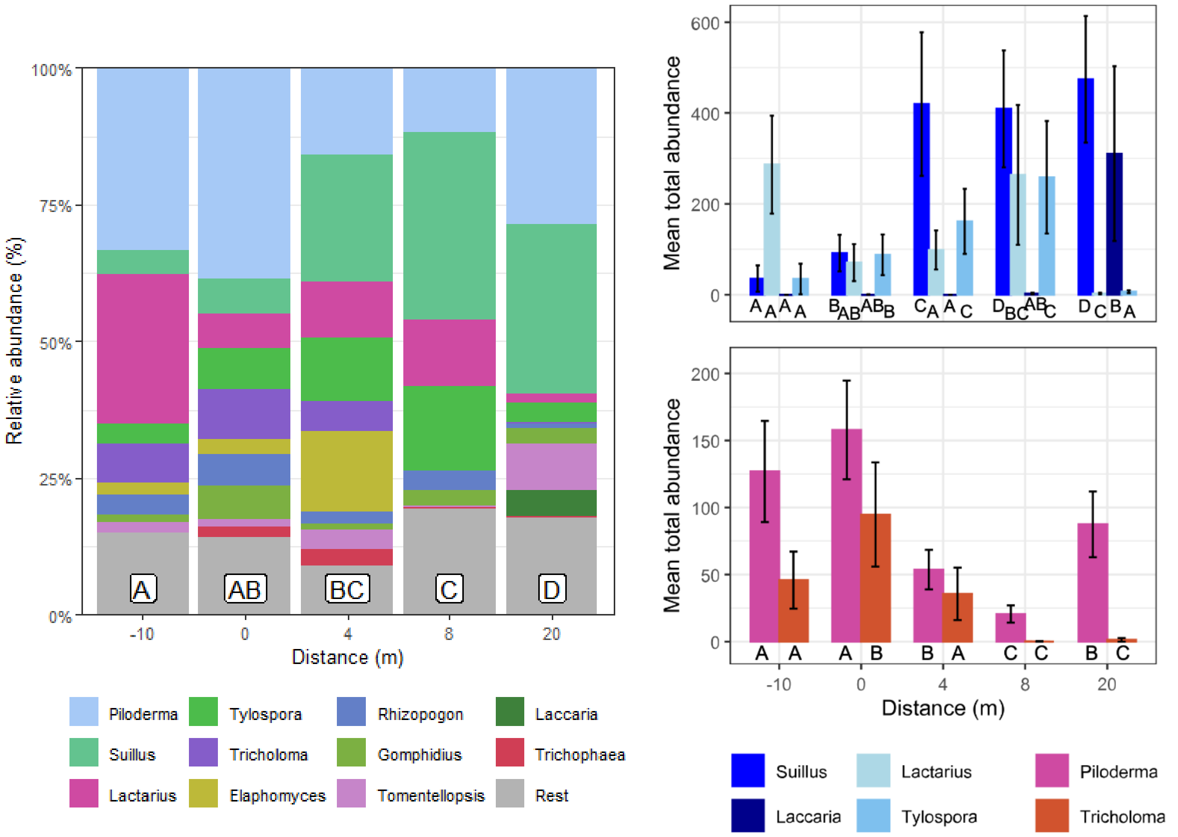


Figure 11. Relative abundance of the 20 most abundant ECM OTUs and the mean abundance of early- (blue) and late-stage (red) genera per distance. As distance from the forest increased, the ECM community composition gradually shifted, early-stage ECMs became more abundant and late-stage ECMs became less abundant. OBS: Laccaria was absent at -10 and 4 m and its p-value was set as 1 (not significant).

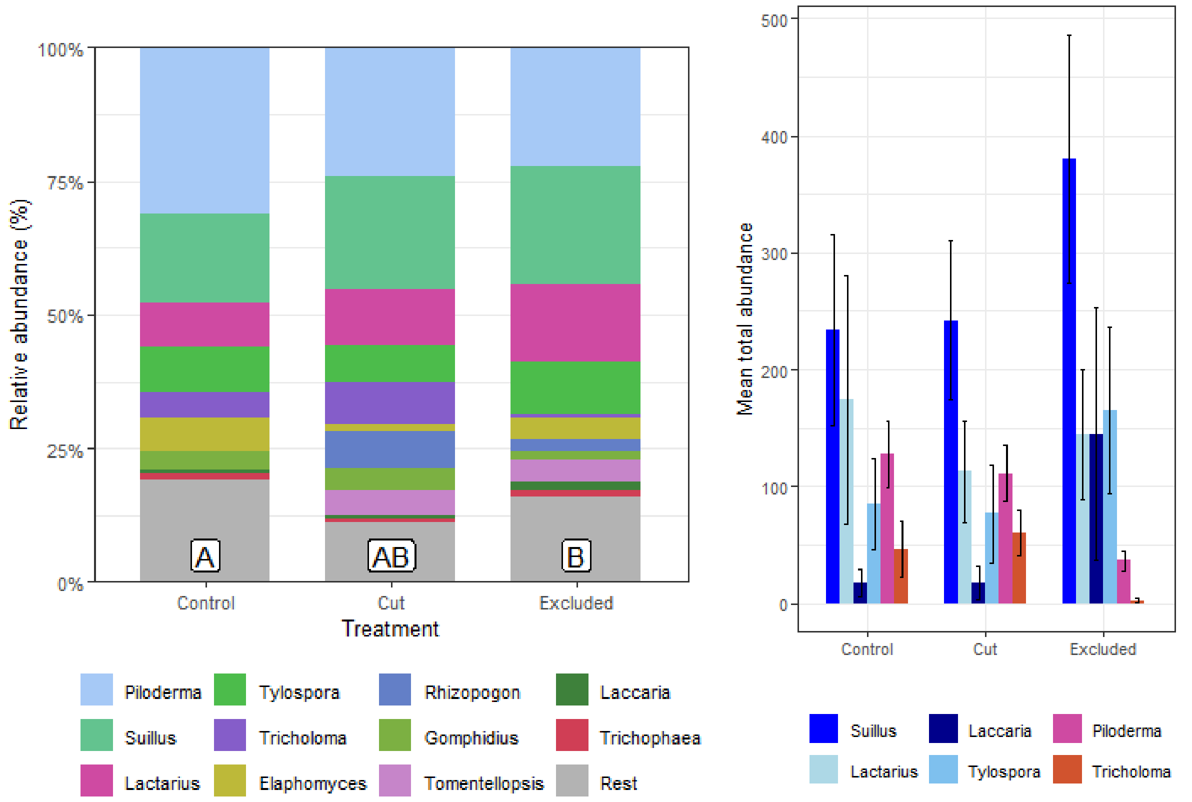


Figure 12. Relative abundance of the 20 most abundant ECM OTUs and the mean abundance of early- (blue) and late-stage (red) genera per treatment. The community composition significantly differed between control and excluded seedlings. Late-stage ECMs were significantly less abundant on excluded seedlings compared to control and cut.

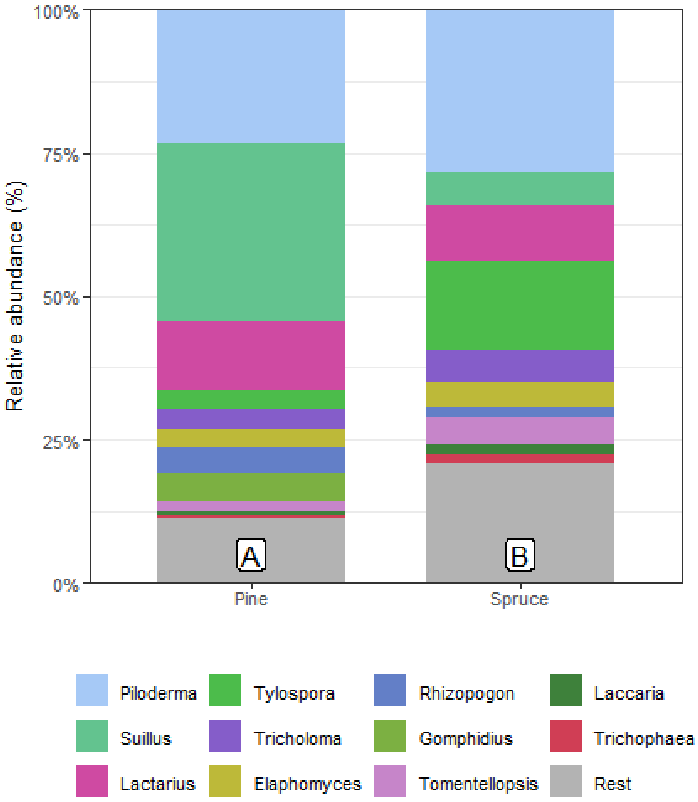


Figure 13. Relative abundance of the 20 most abundant ECM OTUs on pine and spruce seedlings. The two communities significantly differ from each other with Suillus being a common ECM on pine and Tylospora being common on spruce seedlings.

Community diversity

As seedlings got closer to the forest, their ECM community diversity and evenness typically decreased (analyses were done on OTUs). Root excluded seedlings always significantly decreased ($p \leq 0{,}0156$), cut seedlings only significantly decreased regarding the Shannon index ($p = 0{,}0476$), and the remaining samples only showed non-significant trends (fig. 14).

Both the seedlings' distance to the forest edge and its treatment significantly affected their associated ECM community diversity and evenness (fig. 15, $p \leq 0{,}0326$ and $p \leq 0{,}0009$, respectively). Seedlings growing at -10 and 8 m had a lower Shannon and Simpson diversity indexes ($p_{adj} \leq 0{,}0460$) than seedlings growing at 20 m. The ECM species evenness was significantly higher at 20 m compared to the rest (fig. 15a, $p_{adj} \leq 0{,}0444$). ECM community associated with cut seedlings had higher diversity indexes and evenness than control and root excluded seedlings (fig. 15b, $p_{adj} \leq 0{,}0058$).

The seedlings’ dry weight had very little and no significant correlation with any diversity parameters ($p \geq 0{,}3159$ and a Pearson’s correlation estimate $\leq 8{,}5$ %). Regression of the parameters showed low goodness-of-fit values ($R^2 \leq 0{,}0038$) with no significant effect (table S12, $p \geq 0{,}305$).

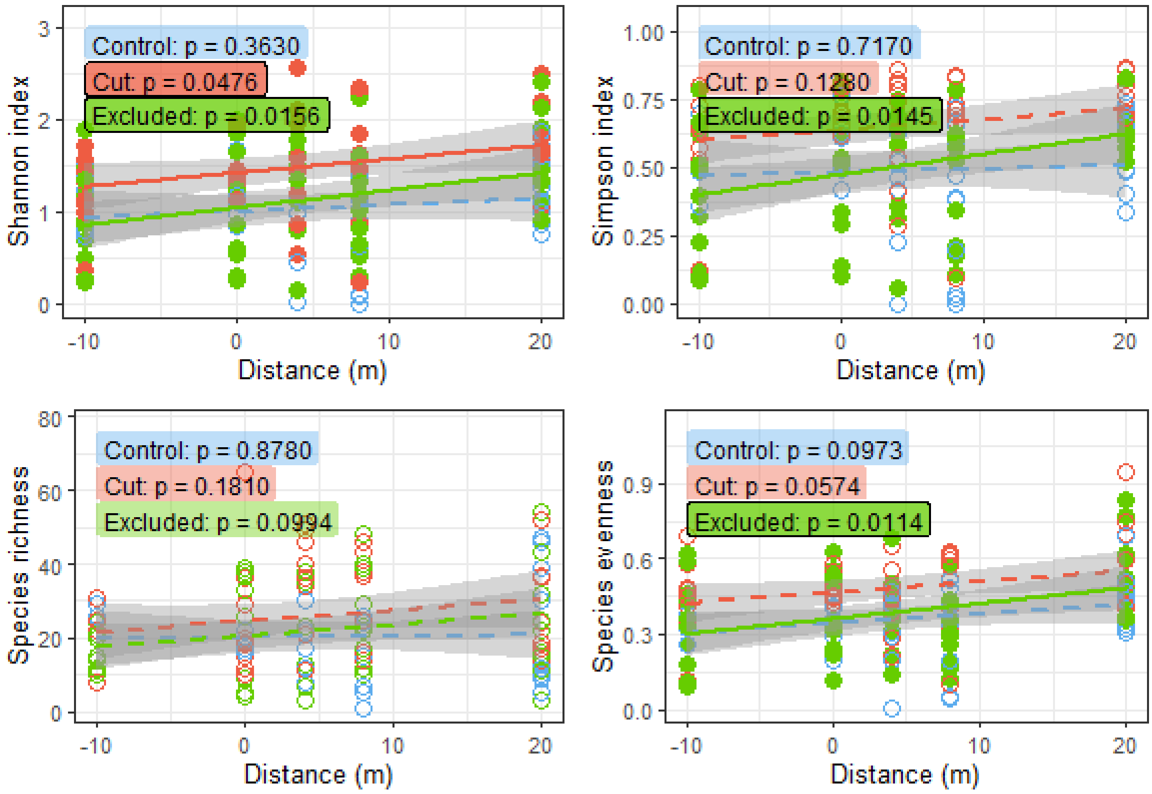
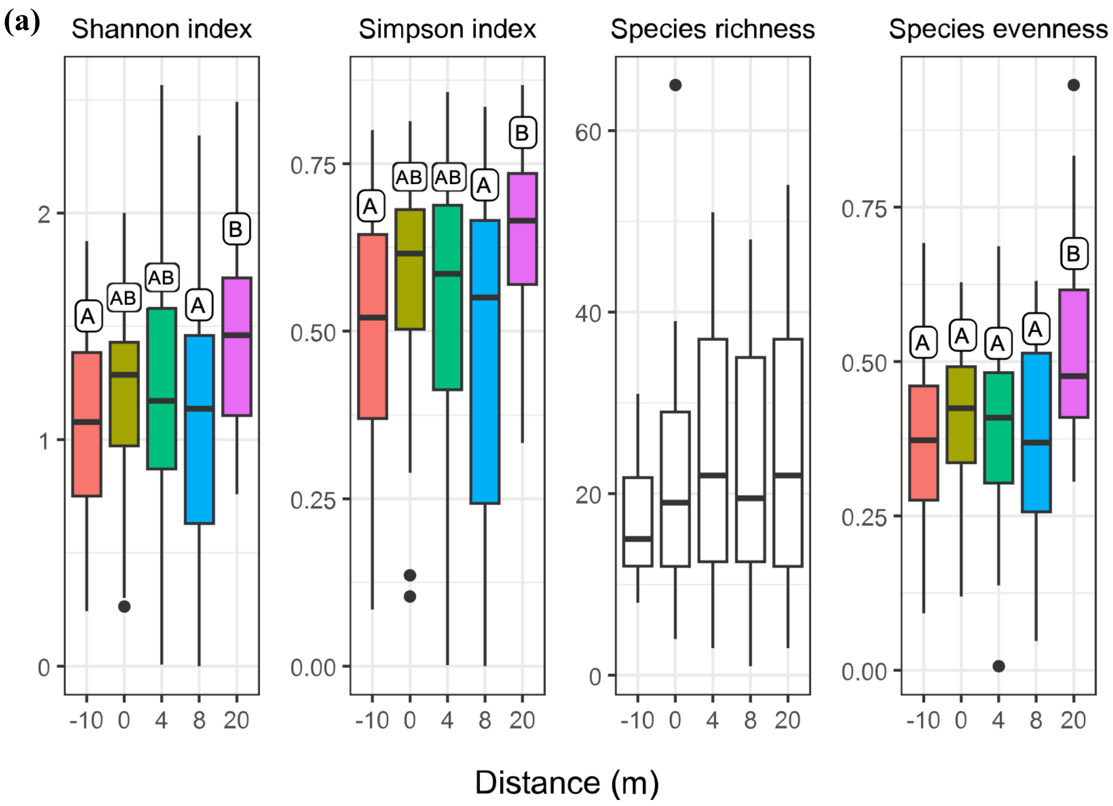


Figure 14. Linear regression of the ECM diversity parameters per treatment across distance to the forest edge. Root excluded seedlings showed the highest response with significantly lower ECM diversity in the forest. Dashed lines with hollow dots are not significant (p > 0,05), while whole lines with filled dots are significant (p ≤ 0,05).



(b)

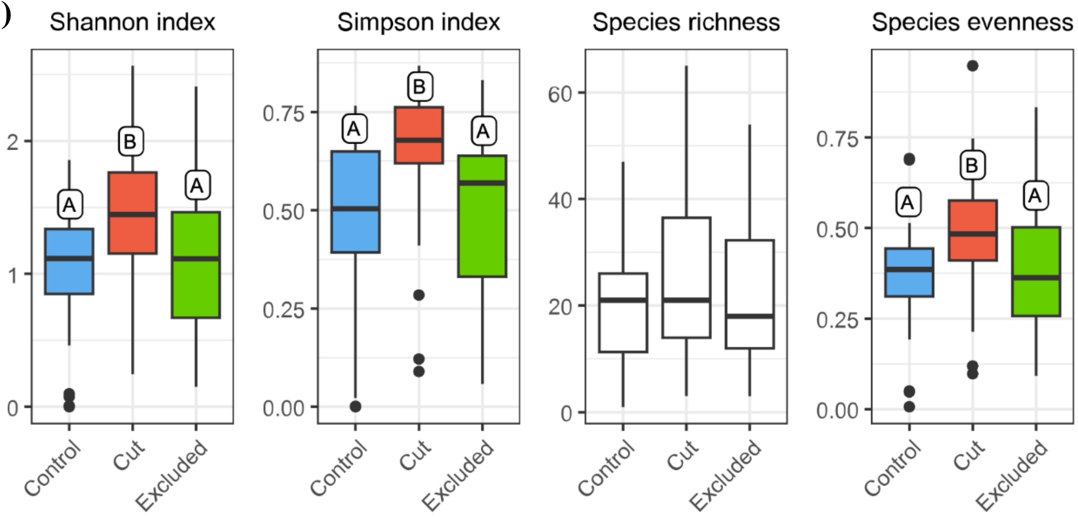


Figure 15. ECM community diversity across distance (a) and treatment (b). Colored plots are parameters where distance or treatment had a significant effect. ECM diversity typically decreased towards the forest, and the cut treatment resulted in the highest diversity.

4. Discussion

This study has brought clear results regarding the edge effect on seedling growth. As distance increased from the forest edge, control and cut seedlings' height, biomass, and N uptake all increased. It not only increased, there is a clear growth threshold between 4 and 8 m corresponding to the reported rooting area of pine trees in the region. Root excluded seedlings were generally bigger but also unaffected by distance, highlighting that belowground N competition is a driver in early seedling growth.

Meanwhile, the underground fungal world shifted along the same gradient. The ECM community composition and diversity varied with distance with early-stage species being more abundant on the clearcut, and late-stage ECMs more abundant in the forest. Late-stage ECM were also significantly more abundant on control seedlings, than on root excluded.

4.1 Seedlings growth and N uptake

Root exclusion effect

Root excluded seedlings height, dry weight and total N content were all greater than control and cut seedlings (fig. 3, 4b). Excluded seedlings weighed at least 3,2 times more and had at least 2,5 more total N than control (fig. 5). Excluded seedlings were also less ^{15}N enriched, showing a greater N uptake (fig. 7). As hypothesized, the benefit from belowground release was bigger in the forest when competing with mature trees. Root excluded seedlings were 8,2 times heavier in the forest, but only 3,2 times heavier on the clearcut compared to control. Needle and root total N content followed the same pattern (fig. 5). My results showed full support of my hypothesis (i) which states: Isolation from belowground connection to surrounding roots and fungal mycelia leads to enhanced seedling growth and N uptake. This effect should be strongest near mature trees, where potential competition is greater.

Häggström et al. (2024) used a 75 cm deep 1,5 m x 1,5 m steel frame to create a root isolated area. Similar to my results, they found that planted root isolated seedlings had greater height and diameter than control on the forest edge and in the forest but were equal to control on the clearcut. Petriţan et al. 2011 found similar effects of enhanced growth when root excluding seedlings from mature trees/overstory via trenching.

Edge effect on growth

My results fully support the second part of my hypothesis (ii), that seedlings growing in or near the forest are smaller than those on the clearcut. Between 4 and 8 m from the forest edge, we see a clear threshold in height, dry weight, and nitrogen content (fig. 3, 4a, 4c). Inside the forest (≤ 4 m), pine and spruce seedlings averaged only about 19,5 cm and 13,3 cm tall, and weighed roughly 2,2 g and 1,3 g, respectively. Beyond 8 m, on the clearcut, pine seedlings reached 27,9 cm and 7,2 g, while spruce reached 18,8 cm and 3,6 g. This growth threshold likely reflects the span of mature pine roots, which previous studies have placed between 3 and 6 m (Jakobsson 2005; Henriksson et al. 2021b; Lutter et al. 2021). Similar reductions in seedling growth within established tree root zones have been reported by several authors (Jakobsson 2005; Axelsson et al. 2014; Häggström et al. 2024).

Biomass and N allocation

My results, however, showed little statistical support for seedlings shifting their root/shoot ratio with distance, but there are patterns. Spruce seedlings showed little plasticity regarding biomass allocation, but pine seedlings root/shoot ratio slightly decreased with distance (significantly for the cut treatment) from ca 0,6 to 0,4 (fig. 6c). Meaning pine seedlings had relatively less root mass on the clearcut than in the forest. Petriţan et al. 2011 found similar results where beech (*Fagus sylvatica*) and Douglas fir (*Pseudotsuga menziesii*) seedlings had less root biomass with increasing light availability and with root exclusion via trenching.

I can only, with caution, provide trends for the first part of my second hypothesis: Seedlings growing in the forest/near mature trees have a higher root/shoot ratio than seedlings growing in the open clearcut.

Surprisingly, excluded seedlings showed the highest relative root mass even though they theoretically had the highest nutrient availability and should then require less root mass. This could be due to the lack of competition to physically limit root growth and perhaps due to the buckets potentially having higher soil moisture content (fig. S13). This could alter root growth (Abramoff & Finzi 2015; Ding et al. 2020; Danzberger et al. 2025). The layout of the experiments site makes it so each transect gets similar sunlight, temperature and precipitation. Soil moisture for the different seedling treatments, however, did differ when measuring two years prior to the harvest of the seedlings. In 2022, during the summer months of June and July, soil moisture was the same for control and excluded seedlings. In august, the moisture content was higher in the buckets probably because there are no trees to absorb it. Meaning after rainfall, the buckets may have retained moisture for a longer period which might have given a boost to the seedlings. Root growth is the most active during these summer months and is thus relevant (Abramoff & Finzi 2015).

Other than the soil moisture potentially being different, I believe that no other major unwanted effects arose within the buckets. Since the soil is native to the site, the amount of nitrogen and other nutrients, as well as the macro- and microscopic flora and fauna communities should have been similar as outside the bucket. Changes in these parameters are then a response to the release from belowground competition or from the disturbance when creating the treatment. Although the soil was carefully dug up to avoid mixing the soil layers and potentially impacting the results.

Results regarding hypothesis (i) and (ii) showcase that the presence of mature trees with an established root system inhibits N uptake and thus growth of seedlings. Figure 4c and 7 showcase that as control and cut seedlings' atom% ^{15}N gets more and more diluted from N uptake, their N content and dry weight increases. Root excluded seedlings' atom% ^{15}N, N content or dry weight however did not vary with distance. With this, I can with confidence state that belowground N competition is a driving limiting factor in seedling growth.

Other insights

Although control and cut pine seedlings on the clearcut had higher N-concentration than excluded seedlings (fig. 8), they were not bigger (fig. 4c). Possibly meaning that the seedlings have taken up N but are unable/unwilling to use it for growth. Perhaps, instead of opting for short-term gains by putting the N to use for height growth, they invest it in their needles for long-term gains by producing higher yielding needles with better photosynthetic capabilities.

Seedlings and their samples from the 4 m mark have on multiple occasions had intermediate values and bridging between the forest and the clearcut, or between control and root excluded seedlings. Their dry weight and root N content was statistically the same as between -10 to 0 m and between 8 and 20 m, their needle N content was the same as between -10 and 0 m and control needles at 8 m (fig. 4c). In addition to this, while not being statistically significant, cut seedlings often had a higher mean dry weight and total N content than control at each distance (fig. 4c). Severing the soil around the seedling and giving it a small grace period without competition seems to have benefited the seedling, sometimes significantly.

This study has looked at 3-year-old seedlings and the effect of belowground competition on their growth and allocations. I hypothesis that this growth dent will persist over a longer period of time, which has been hypothesized or already observed by others (Valkonen et al. 2002; Jakobsson 2005; Axelsson et al. 2014; Henriksson et al. 2023).

Figure 16. Photo showing the growth dent on older seedlings in proximity to the forest edge (photo by Peter Högberg from Henriksson et al. 2023).

4.2 ECM associations

ECM composition shift

Contrary to height, DW and N data, there was no clear threshold in ECM community composition between forest and clearcut between 4 and 8 m, instead my results showed a smoother transition from -10 to 20 m (fig. 11). Perhaps because the reach of different ECM species mycelia can differ greatly and the space ECM species take up is very patchy creating more variation and a smoother look (Douhan et al. 2011). As well as a general ECM shift, there is also a shift between early- to late-stage species. Both groups are present at all distances, but their relative abundance differs. Early ECMs are more abundant on the clearcut, and late ECMs are more abundant closer to, and in, the forest (fig. 11). These findings support my first fungal-related hypothesis which states: The ectomycorrhizal community composition differs between forest and clearcut, with species associated with later successional stages being present in the forest but absent in the clearcut.

My classifications of the ECMs into either early- or late-stage were based on scientific literature, including several factors such as main N uptake source (mineral or organic), response to N addition/elevation, ability to produce oxidizing agents to decompose complex organic matter, hydrophobicity of hyphae, and chronological appearance/dominance. Since the appearance and dominance of ECM species varies depending on the local climate, nutrient, and moisture conditions Jörgensen et al. (2025) instead propose a continuous scale from absorbers of easily accessible mineral N to miners of complex organic matter. While in essence the same thing as successional stages, it is not the actual time itself which determines which genera are present, rather the local conditions which vary over time.

Renaming the early- and late-stage groups to absorbers and miners is better suited and should be done in future research, by myself and by others. I suspect that as an additional tool to determine whether the ECM community is composed of more absorbers or miners is to measure the enzyme activity in the soil. The concentration of decomposing/oxidizing enzymes should correlate with an increasing abundance of miner genera which should help determine the state of the community along this absorber-miner gradient.

I first considered the use and analysis of exploration types of the ECMs and how they vary over the forest-clearcut transect. While exploration types can provide information about the ecology of a certain genus, it can also be misleading.

For example, both *Suillus* and *Cortinarius* have long-distance hyphae which can travel long distances (Agerer 2001; Lilleskov et al. 2011; van der Linde et al. 2018; Clemmensen et al. 2021; Jörgensen et al. 2023, 2025). However, *Suillus* is a well-known pioneer and early colonizer and so it might use its long-distance hyphae to spread wide in search for new seedlings to colonize. *Cortinarius*, on the other hand, is a well-known N-miner and late-stage genus which typically dominate in mature forests and might instead use its long-distance hyphae to search for nutrients (Kyaschenko et al. 2017; Clemmensen et al. 2021; Jörgensen et al. 2025).

ECM across treatments

While the composition difference doesn't change much visually in the top 20 ECM OTUs, the PERMANOVA showed a significant difference (fig. 12). Late-stage genera were significantly more abundant on control and cut than on root excluded seedlings (fig. 12). I can reject my hypothesis (iv) which states: Root isolation does not affect ectomycorrhizal species composition.

Supposedly, there were more nutrients available within the bucket since there are only two seedlings competing with each other, rather than the whole belowground agglomeration of tree roots and their extensive fungi mycelia. This should theoretically let early-stage ECMs retain their abundance for longer while late-stage miners are outcompeted until nutrients become scarce enough for miners to be competitive. As stated previously, the buckets had a higher soil moisture content which has been observed to affect the fungal community on beech and oak stands (Buée et al. 2005; Flores-Rentería et al. 2016).

Another theory is that the ECM composition is influenced by the degree of disturbance. Although not the intended goal, rather the opposite, digging up the soil to put in the buckets caused a disturbance which could have disrupted the establishment and colonization of late-stage ECMs. A smaller disturbance, such as severing the soil surrounding the seedling or simply planting the seedling allowed late-stage ECMs to persist and retain abundance.

It's probably a combination of the explanations above. The root exclusion treatment created the greatest disturbance and had the highest available amounts of nutrients leading to more early-stage and pioneering ECMs. Severing the soil around the seedling also provided a short grace period possibly delaying the ingrowth of outside ECMs and roots, allowing early-stage ECMs to retain abundance for a little longer.

Other findings

Results from the community diversity analyses across distance and treatment were unexpected (fig. 14, 15). Contrary to my expectations, control seedlings ECM community diversity parameters were unaffected by their distance to the forest edge, but root excluded seedlings showed the highest response (lower values in/near the forest), cut seedlings expressed significantly higher values for Shannon, Simpson and evenness. In the forest, fewer ECM OTUs dominate the root tips of excluded seedlings (Shannon, Simpson and evenness significantly decrease). This trend is true for cut seedlings as well but not statistically supported except for the Shannon index.

4.3 It takes two to tango

My findings do not support my final hypothesis which states: Bigger seedlings have greater ectomycorrhizal species diversity. Contrary to my expectations, the weight of the seedling had no correlation with their associated ECM diversity. The diversity parameters I used (Shannon and Simpson index, species richness and evenness) only measure the number of species and their relative abundance and don't account for which specific genera are present. Community composition, however, did differ between the sizes, but that variation and shift is already explained by the seedlings' distance to the forest. Explaining the variation in size among seedlings of the same age and similar environmental conditions is challenging. Local nutrient availability, microclimate, and seedling genetics likely play important roles.

Seedlings and their associated ECM communities form an interconnected network that grows and shifts simultaneously as environmental conditions change. Determining whether one system initiates change or if both are shifting hand-in-hand is difficult, resembling the “chicken or the egg” scenario. As seedlings grow, their ECM-assisted roots progressively deplete easily available nitrogen, prompting a shift in the ECM community toward types specialized in nitrogen mining. This shift enables further growth, establishing a positive feedback loop.

However, seedlings do not actively select their fungal partners; instead, fungi compete among themselves for colonization opportunities and for nutrient uptake. In a forest, where nutrients are scarce and locked in complex organic matter, miner type ECMs will be more competitive and thus colonize more of the seedling’s roots. On a clearcut, however, following the removal of trees and the decomposition of fresh litter by saprotrophs, nitrogen will be easily accessible in a higher supply, inviting absorber type ECMs to thrive and thus colonize the seedlings.

Thus, the observed association between seedlings size and associated ECM is likely coincidental rather than causal, reflecting seedling and ECM responding to the same environmental factors rather than a direct cause-and-effect relationship.

I have only analyzed the ECM community on seedlings, and refrained from analyzing saprotrophs, parasites, or other forms of mycorrhizal fungi. However, various fungi predators can exert significant pressure altering the community composition and affecting the performance of the fungi (Moore et al. 2003).While the soil community is primarily determined by resource availability, rather than predation, it could be worthwhile to examine or at least keep in mind (Moore et al. 2003; van der Heyde et al. 2019:2021; Pérez-Izquierdo et al. 2021).

4.4 Broader view and implications for forestry

This study has analyzed seedlings growing on a dry, sandy and relatively poor site with mature pine trees, and has thus mainly been compared to studies with similar conditions. While these site conditions are common in Sweden, especially in the northern parts, my findings and conclusions should then primarily be applied to similar sites. My conclusion that belowground competition for nitrogen hampers seedling growth in the edge zone implies that the edge effect should be stronger on poorer sites and less noticeable on more fertile sites. However, the net effect on seedlings is presumably an interaction of competition for nutrients and water, and shading by overstory trees, and experiments such as this one should then be carried out across Sweden at different site conditions to get a better understanding of the limitations for seedling growth.

My findings are of importance to Swedish forestry, especially if clearcuts are going to be limited in size. While setting a limit on clearcut sizes can be a reasonable change in order to mitigate some of the negative impacts of forestry, the change must be made knowing that it will affect the growth of seedlings, and ultimately the production of timber. My research was on acquiring further knowledge of the edges, not on practical ways of minimizing the effect. With that said, simply severing the soil surrounding the seedlings seemed to have promoted a slight growth increase (fig. 4b). Further research on more practical and viable ways of creating a barrier could be crucial for sustaining wood production and still getting the ecological benefits of smaller clearcuts.

References

Abramoff, R.Z. & Finzi, A.C. (2015). Are above- and below-ground phenology in sync? *New Phytologist*, 205 (3), 1054–1061. https://doi.org/10.1111/nph.13111

Agerer, R. (2001). Exploration types of ectomycorrhizae. *Mycorrhiza*, 11 (2), 107–114. https://doi.org/10.1007/s005720100108

Axelsson, E. & Axelsson, B. (1986). Changes in carbon allocation patterns in spruce and pine trees following irrigation and fertilization. *Tree Physiology*, 2 (1-2–3), 189–204. https://doi.org/10.1093/treephys/2.1-2-3.189

Axelsson, E.P., Lundmark, T., Högberg, P. & Nordin, A. (2014). Belowground Competition Directs Spatial Patterns of Seedling Growth in Boreal Pine Forests in Fennoscandia. *Forests*, 5 (9), 2106–2121. https://doi.org/10.3390/f5092106

Becquer, A., Guerrero-Galán, C., Eibensteiner, J.L., Houdinet, G., Bücking, H., Zimmermann, S.D. & Garcia, K. (2019). The ectomycorrhizal contribution to tree nutrition. In: *Advances in Botanical Research*. Elsevier. 77–126. https://doi.org/10.1016/bs.abr.2018.11.003

Björs, M. (2024). Gap-edge Effects on Seedling Growth, N Uptake and Associated Ectomycorrhizal Fungi.

Bödeker, I.T.M., Clemmensen, K.E., de Boer, W., Martin, F., Olson, Å. & Lindahl, B.D. (2014). Ectomycorrhizal Cortinarius species participate in enzymatic oxidation of humus in northern forest ecosystems. *The New Phytologist*, 203 (1), 245–256. https://doi.org/10.1111/nph.12791

Buée, M., Vairelles, D. & Garbaye, J. (2005). Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (Fagus silvatica) forest subjected to two thinning regimes. *Mycorrhiza*, 15 (4), 235–245. https://doi.org/10.1007/s00572-004-0313-6

Bunn, R.A., Corrêa, A., Joshi, J., Kaiser, C., Lekberg, Y., Prescott, C.E., Sala, A. & Karst, J. (2024). What determines transfer of carbon from plants to mycorrhizal fungi? *New Phytologist*, 244 (4), 1199–1215. https://doi.org/10.1111/nph.20145

Cambui, C.A., Svennerstam, H., Gruffman, L., Nordin, A., Ganeteg, U. & Näsholm, T. (2011). Patterns of Plant Biomass Partitioning Depend on Nitrogen Source. *PLoS ONE*, 6 (4), e19211. https://doi.org/10.1371/journal.pone.0019211

Clemmensen, K.E., Durling, M.B., Michelsen, A., Hallin, S., Finlay, R.D. & Lindahl, B.D. (2021). A tipping point in carbon storage when forest expands into tundra is related to mycorrhizal recycling of nitrogen. *Ecology Letters*, 24 (6), 1193–1204. https://doi.org/10.1111/ele.13735

Clemmensen, K.E., Finlay, R.D., Dahlberg, A., Stenlid, J., Wardle, D.A. & Lindahl, B.D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, 205 (4), 1525–1536. https://doi.org/10.1111/nph.13208

Danzberger, J., Hikino, K., Landhäusser, S.M., Hesse, B.D., Meyer, S., Buegger, F., Weikl, F., Grams, T.E.E. & Pritsch, K. (2025). Drought acclimation of beech seedlings depends largely on their rooting patterns and less on the fungal communities in soils. *Plant and Soil*, 508 (1), 49–70. https://doi.org/10.1007/s11104-024-06784-7

Ding, Y., Schiestl-Aalto, P., Helmisaari, H.-S., Makita, N., Ryhti, K. & Kulmala, L. (2020). Temperature and moisture dependence of daily growth of Scots pine (Pinus sylvestris L.) roots in Southern Finland. *Tree Physiology*, 40 (2), 272–283. https://doi.org/10.1093/treephys/tpz131

Douhan, G.W., Vincenot, L., Gryta, H. & Selosse, M.-A. (2011). Population genetics of ectomycorrhizal fungi: from current knowledge to emerging directions. *Fungal Biology*, 115 (7), 569–597. https://doi.org/10.1016/j.funbio.2011.03.005

Flores-Rentería, D., Rincón, A., Valladares, F. & Curiel Yuste, J. (2016). Agricultural matrix affects differently the alpha and beta structural and functional diversity of soil microbial communities in a fragmented Mediterranean holm oak forest. *Soil Biology and Biochemistry*, 92, 79–90. https://doi.org/10.1016/j.soilbio.2015.09.015

Forest Act for Baden-Württemberg (1996). . https://www.landesrecht-bw.de/bsbw/document/jlr-WaldGBWpP15 [2025-05-22]

From, F. (2014). *Long-term effects of nitrogen (N) fertilizer and simulated N deposition on boreal forest growth*. https://res.slu.se/id/publ/54917 [2025-01-31]

Fry, B. (2006). *Stable Isotope Ecology*. Springer. https://doi.org/10.1007/0-387-33745-8

Gruffman, L., Ishida, T., Nordin, A. & Näsholm, T. (2012). Cultivation of Norway spruce and Scots pine on organic nitrogen improves seedling morphology and field performance. *Forest Ecology and Management*, 276, 118–124. https://doi.org/10.1016/j.foreco.2012.03.030

Häggström, B., Gundale, M.J. & Nordin, A. (2024). Environmental controls on seedling establishment in a boreal forest: implications for Scots pine regeneration in continuous cover forestry. *European Journal of Forest Research*, 143 (1), 95–106. https://doi.org/10.1007/s10342-023-01609-1

Henriksson, N., Franklin, O., Tarvainen, L., Marshall, J., Lundberg-Felten, J., Eilertsen, L. & Näsholm, T. (2021a). The mycorrhizal tragedy of the commons. *Ecology Letters*, 24 (6), 1215–1224. https://doi.org/10.1111/ele.13737

Henriksson, N., Lim, H., Marshall, J., Franklin, O., McMurtrie, R.E., Lutter, R., Magh, R., Lundmark, T. & Näsholm, T. (2021b). Tree water uptake enhances nitrogen acquisition in a fertilized boreal forest – but not under nitrogen-poor conditions. *New Phytologist*, 232 (1), 113–122. https://doi.org/10.1111/nph.17578

Henriksson, N., Marshall, J., Högberg, M.N., Högberg, P., Polle, A., Franklin, O. & Näsholm, T. (2023). Re-examining the evidence for the mother tree hypothesis – resource sharing among trees via ectomycorrhizal networks. *New Phytologist*, 239 (1), 19–28. https://doi.org/10.1111/nph.18935

van der Heyde, M., Abbott, L.K., Gehring, C., Kokkoris, V. & Hart, M.M. (2019). Reconciling disparate responses to grazing in the arbuscular mycorrhizal symbiosis. *Rhizosphere*, 11, 100167. https://doi.org/10.1016/j.rhisph.2019.100167

Jakobsson, R. (2005). Growth of Retained Scots Pines and Their Influence on the New Stand.

Jörgensen, K., Clemmensen, K.E., Fransson, P., Manzoni, S., Wallander, H. & Lindahl, B.D. (2025). A trait spectrum linking nitrogen acquisition and carbon use of ectomycorrhizal fungi. *New Phytologist*,. https://doi.org/10.1111/nph.70129

Jörgensen, K., Clemmensen, K.E., Wallander, H. & Lindahl, B.D. (2023). Do ectomycorrhizal exploration types reflect mycelial foraging strategies? *New Phytologist*, 237 (2), 576–584. https://doi.org/10.1111/nph.18566

Kyaschenko, J., Clemmensen, K.E., Hagenbo, A., Karltun, E. & Lindahl, B.D. (2017). Shift in fungal communities and associated enzyme activities along an age gradient of managed Pinus sylvestris stands. *The ISME Journal*, 11 (4), 863–874. https://doi.org/10.1038/ismej.2016.184

Länstyrelsen (n.d.). *Virkesförråd fördelad på trädslag och ålderklasser. Länsstyrelsernas statistik.* https://pxweb.lansstyrelsen.se:443/pxweb/sv/Miljotillstandet_i_skogslandskapet/Miljotillstandet_i_skogslandskapet__VirkesforradTradslagAlder/Volym_tradslag.px/ [2025-01-30]

Lehto, T. & Zwiazek, J.J. (2011). Ectomycorrhizas and water relations of trees: a review. *Mycorrhiza*, 21 (2), 71–90. https://doi.org/10.1007/s00572-010-0348-9

Lilleskov, E.A., Hobbie, E.A. & Horton, T.R. (2011). Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology*, 4 (2), 174–183. https://doi.org/10.1016/j.funeco.2010.09.008

van der Linde, S., Suz, L.M., Orme, C.D.L., Cox, F., Andreae, H., Asi, E., Atkinson, B., Benham, S., Carroll, C., Cools, N., De Vos, B., Dietrich, H.-P., Eichhorn, J., Gehrmann, J., Grebenc, T., Gweon, H.S., Hansen, K., Jacob, F., Kristöfel, F., Lech, P., Manninger, M., Martin, J., Meesenburg, H., Merilä, P., Nicolas, M., Pavlenda, P., Rautio, P., Schaub, M., Schröck, H.-W., Seidling, W., Šrámek, V., Thimonier, A., Thomsen, I.M., Titeux, H., Vanguelova, E., Verstraeten, A., Vesterdal, L., Waldner, P., Wijk, S., Zhang, Y., Žlindra, D. & Bidartondo, M.I. (2018). Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature*, 558 (7709), 243–248. https://doi.org/10.1038/s41586-018-0189-9

Linder, S. & Troeng, E. (1980). Photosynthesis and Transpiration of 20-Year-Old Scots Pine. *Ecological Bulletins*, (32), 165–181

Lutter, R., Henriksson, N., Lim, H., Blaško, R., Magh, R.-K., Näsholm, T., Nordin, A., Lundmark, T. & Marshall, J.D. (2021). Belowground resource utilization in monocultures and mixtures of Scots pine and Norway spruce. *Forest Ecology and Management*, 500, 119647. https://doi.org/10.1016/j.foreco.2021.119647

Ma, S., He, F., Tian, D., Zou, D., Yan, Z., Yang, Y., Zhou, T., Huang, K., Shen, H. & Fang, J. (2018). Variations and determinants of carbon content in plants: a global synthesis. *Biogeosciences*, 15 (3), 693–702. https://doi.org/10.5194/bg-15-693-2018

Mahmood, S., Finlay, R.D., Wallander, H. & Erland, S. (2002). Ectomycorrhizal colonisation of roots and ash granules in a spruce forest treated with granulated wood ash. *Forest Ecology and Management*, 160 (1), 65–74. https://doi.org/10.1016/S0378-1127(01)00462-5

Martin, F., Duplessis, S., Ditengou, F., Lagrange, H., Voiblet, C. & Lapeyrie, F. (2001). Developmental cross talking in the ectomycorrhizal symbiosis: signals and communication genes. *New Phytologist*, 151 (1), 145–154. https://doi.org/10.1046/j.1469-8137.2001.00169.x

Moore, J.C., McCann, K., Setälä, H. & De Ruiter, P.C. (2003). Top-down Is Bottom-up: Does Predation in the Rhizosphere Regulate Aboveground Dynamics? *Ecology*, 84 (4), 846–857. https://doi.org/10.1890/0012-9658(2003)084[0846:TIBDPI]2.0.CO;2

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S.G., Campbell, C., Hurry, V., Linder, S. & Högberg, M.N. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *The New Phytologist*, 198 (1), 214–221

New EU Forest Strategy for 2030 (2021). . https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52021DC0572 [2025-01-28]

Nohrstedt, H.-Ö. (2001). Response of Coniferous Forest Ecosystems on Mineral Soils to Nutrient Additions: A Review of Swedish Experiences. *Scandinavian Journal of Forest Research*, 16 (6), 555–573. https://doi.org/10.1080/02827580152699385

Pérez-Izquierdo, L., Rincón, A., Lindahl, B.D. & Buée, M. (2021). Chapter 13 - Fungal community of forest soil: Diversity, functions, and services. In: Asiegbu, F.O. & Kovalchuk, A. (eds) *Forest Microbiology*. Academic Press. 231–255. https://doi.org/10.1016/B978-0-12-822542-4.00022-X

Petriţan, I.C., von Lüpke, B. & Petriţan, A.M. (2011). Effects of root trenching of overstorey Norway spruce (Piceaabies) on growth and biomass of underplanted beech (Fagussylvatica) and Douglas fir (Pseudotsugamenziesii) saplings. *European Journal of Forest Research*, 130 (5), 813–828. https://doi.org/10.1007/s10342-010-0473-1

Põlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kauserud, H., Nguyen, N., Kjøller, R., Bates, S.T., Baldrian, P., Frøslev, T.G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järv, H., Madrid, H., Nordén, J., Liu, J.-K., Pawlowska, J., Põldmaa, K., Pärtel, K., Runnel, K., Hansen, K., Larsson, K.-H., Hyde, K.D., Sandoval-Denis, M., Smith, M.E., Toome-Heller, M., Wijayawardene, N.N., Menolli, N., Reynolds, N.K., Drenkhan, R., Maharachchikumbura, S.S.N., Gibertoni, T.B., Læssøe, T., Davis, W., Tokarev, Y., Corrales, A., Soares, A.M., Agan, A., Machado, A.R., Argüelles-Moyao, A., Detheridge, A., de Meiras-Ottoni, A., Verbeken, A., Dutta, A.K., Cui, B.-K., Pradeep, C.K., Marín, C., Stanton, D., Gohar, D., Wanasinghe, D.N., Otsing, E., Aslani, F., Griffith, G.W., Lumbsch, T.H., Grossart, H.-P., Masigol, H., Timling, I., Hiiesalu, I., Oja, J., Kupagme, J.Y., Geml, J., Alvarez-Manjarrez, J., Ilves, K., Loit, K., Adamson, K., Nara, K., Küngas, K., Rojas-Jimenez, K., Bitenieks, K., Irinyi, L., Nagy, L.G., Soonvald, L., Zhou, L.-W., Wagner, L., Aime, M.C., Öpik, M., Mujica, M.I., Metsoja, M., Ryberg, M., Vasar, M., Murata, M., Nelsen, M.P., Cleary, M., Samarakoon, M.C., Doilom, M., Bahram, M., Hagh-Doust, N., Dulya, O., Johnston, P., Kohout, P., Chen, Q., Tian, Q., Nandi, R., Amiri, R., Perera, R.H., dos Santos Chikowski, R., Mendes-Alvarenga, R.L., Garibay-Orijel, R., Gielen, R., Phookamsak, R., Jayawardena, R.S., Rahimlou, S., Karunarathna, S.C., Tibpromma, S., Brown, S.P., Sepp, S.-K., Mundra, S., Luo, Z.-H., Bose, T., Vahter, T., Netherway, T., Yang, T., May, T., Varga, T., Li, W., Coimbra, V.R.M., de Oliveira, V.R.T., de Lima, V.X., Mikryukov, V.S., Lu, Y., Matsuda, Y., Miyamoto, Y., Kõljalg, U. & Tedersoo, L. (2020). FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity*, 105 (1), 1–16. https://doi.org/10.1007/s13225-020-00466-2

Smith, S.E. & Read, D. (2008). 15 - The roles of mycorrhizas in successional processes and in selected biomes. In: Smith, S.E. & Read, D. (eds) *Mycorrhizal Symbiosis (Third Edition)*. Academic Press. 525–572. https://doi.org/10.1016/B978-012370526-6.50017-9

Tamm, C.O. (1991). *Nitrogen in Terrestrial Ecosystems*. Springer. https://doi.org/10.1007/978-3-642-75168-4

The European Green Deal - European Commission (2024). https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/story-von-der-leyen-commission/european-green-deal_en [2025-01-28]

Valkonen, S., Ruuska, J. & Siipilehto, J. (2002). Effect of retained trees on the development of young Scots pine stands in Southern Finland. *Forest Ecology and Management*, 166 (1), 227–243. https://doi.org/10.1016/S0378-1127(01)00668-5

Werner, R.A., Bruch, B.A. & Brand, W.A. (1999). ConFlo III – an interface for high precision δ13C and δ15N analysis with an extended dynamic range. *Rapid Communications in Mass Spectrometry*, 13 (13), 1237–1241. https://doi.org/10.1002/(SICI)1097-0231(19990715)13:13<1237::AID-RCM633>3.0.CO;2-C

Popular science summary

Forests are complex ecosystems where invisible partnerships along-side fierce competition takes place beneath our feet. One of the most important of these partnerships is the symbiosis between trees and mycorrhizal fungi. The mycorrhizal fungi extend upon the tree roots to provide more nutrient and water to the tree, while the tree provides sugars through its photosynthesis. The fierce competition is for the limiting growth factor, nitrogen, where trees and fungi have evolved their own strategies to acquire this necessity.

In this study, I explored how forestry, specifically clearcuts, affects this partnership and competition. Pine and spruce seedlings from northern Sweden growing at different distances from a forest edge were dug up at examined. Some seedlings were grown inside buckets to block root contact in order to further evaluate influences of the belowground. By weighing different parts of the seedling and using nitrogen isotopes and DNA sequencing, I was able to analyze how much each seedling had grown, how much nitrogen was taken up and where it was allocated, and what kind of symbionts the seedlings partnered up with.

I found that seedlings with less belowground competition, either from growing in a bucket or by leaving the competitive forest, grew bigger and better and hosted different kinds of symbionts. The seedlings growing in the older forest were colonized by late-stage mycorrhizae specialized in mining for nutrient in a competitive arena, while the seedlings on the clearcut had early-stage symbionts which were better at colonizing and absorbing abundant nutrients released after the clearcut. My results show that soil disturbance and competing neighbors strongly shape how seedlings perform, and which symbionts are present.

This study has highlighted that forest edges are more than just a line on a map. Understanding how fungi and tree seedlings interact in these transition zones can help us manage forests more sustainably, especially as European forestry policy shifts toward more ecological focus, favoring either continuous cover forestry or smaller clearcut sizes which create proportionally more forest edge.

Acknowledgement

I would like to thank **Nils Henriksson** for his excellent supervision throughout my project. Your feedback, steady support and curiosity guided me in shaping this thesis.

I am also grateful to **Marcus Björs** and **Jasmin Danzberger** for their valuable assistance in the laboratory, specifically with the weighing and grinding of seedlings and conducting DNA extractions.

Supplementary information

Method to correct for soil contaminated root samples

Initial results from the isotopic analysis showed a suspiciously lower C-concentration in the roots from transect A and B. This is most probably due to bad handling of the samples in the field where the roots were not properly cleaned from all dirt. These mineral particles then stayed all throughout weighing and C/N isotope analysis. To correct this error, simple recalculations were made:

$$New\ corrected\ DW = DW\ of\ contaminated\ sample \times correction\ ratio$$

Where:

$$Correction\ ratio = \frac{\%C\ contaminated\ sample\ -\ \%C\ soil}{\%C\ clean\ root\ -\ \%C\ soil}$$

Here *%C contaminated sample* is the C-concentration in the sample collected from the field with soil remaining on the root, *%C Soil* is assumed to be 0 %, and *%C clean root* is the average C-concentration from the transects C-H which is 48,15 %.

There might be C in the mineral particles but the usual process to determine the C-concentration in samples is to incinerate them. During this process, the organic material combusts and the C is released and measured while the inorganic soil material remains intact preserving the C making it unmeasured. The root samples from transect C-H might also have some soil on them, although negligible since the C-concentration were 48,15 % and should be approximately 48,43 ± 4,16 % in roots (Ma et al. 2018).

The new DW decreases the mean weight in transect A (from 2 g to 1,5 g) and slightly in transect B (1,23 g to 1,14 g) but more importantly, this correction removed the effect of transects on the root/shoot ratio.

The C-concentration for transect A and B were brought up to 48,15 % unless the original value was higher and thus kept. Meaning that for transect A and B, there is a hard bottom cap at 48,15 % with very little variation which only varies upwards.

Using the same correction ratio, new N-concentrations were also calculated for the roots in transect A and B.

$$New\ corrected\ \%N = \%N\ contaminated\ sample * (1 + (1 - correction\ ratio))$$

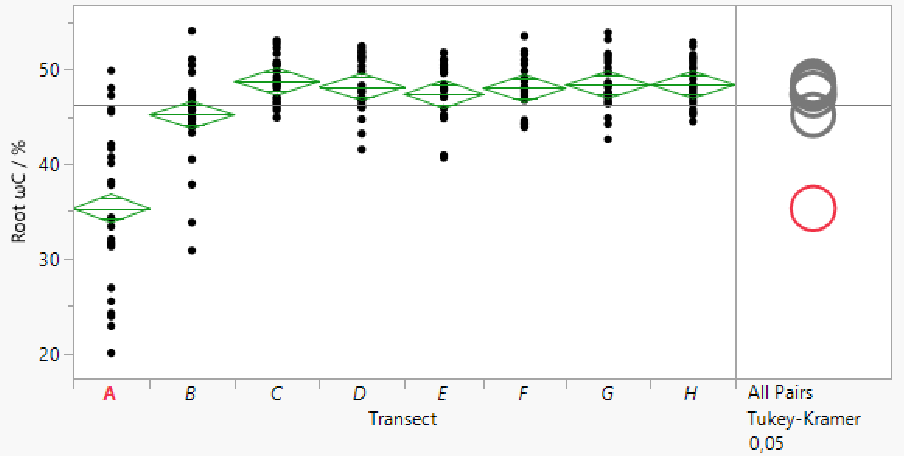


Figure S1. Root N concentration prior to corrections. Lower, and much lower, values for transect B and A.

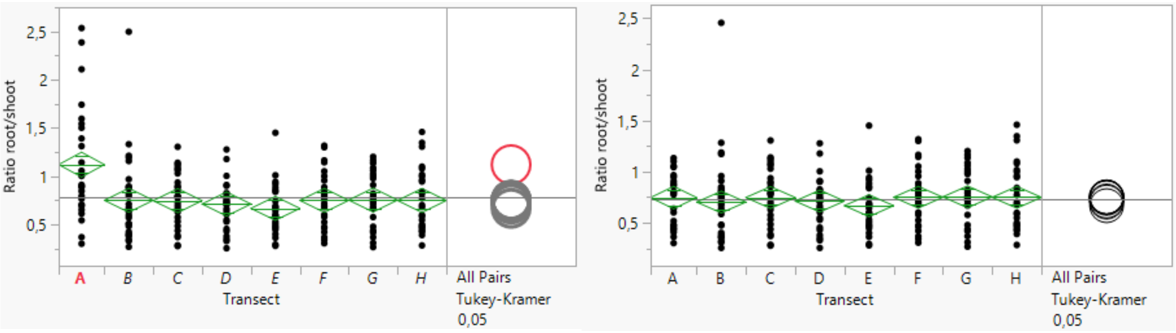


Figure S2. Root/shoot ratio before (left) and after (right) correcting the dry weight in transect A and B.

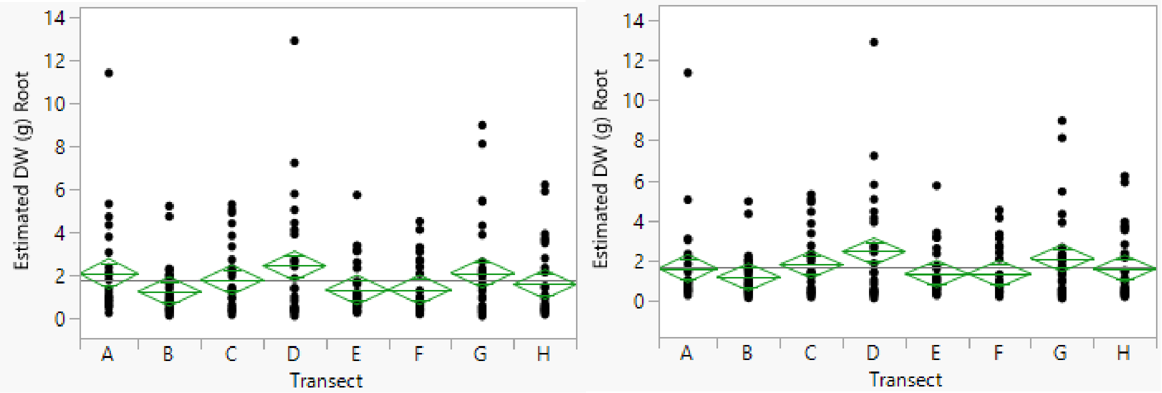


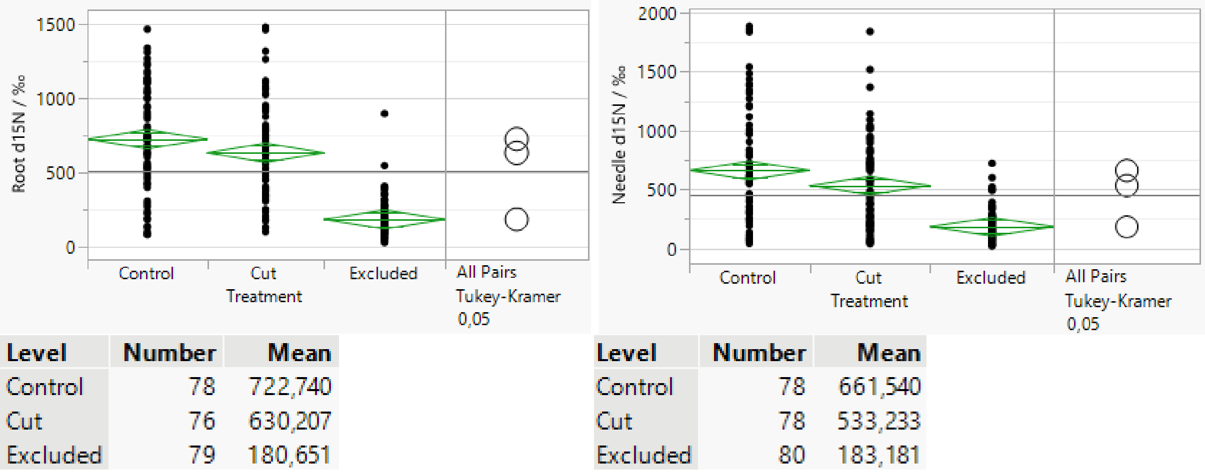
Figure S3. Root dry weight (g) before (left and after (right) correcting the biomass in transect A and B. Lower weights in transect A and B but not significantly different from the remaining transects.

Method to correct for isotope measurement error

An unavoidable flaw with the isotope measurement method is that for each measurement, the next one will be ever so slightly contaminated from the previous one. This contamination is usually not noticeable for samples with natural abundance and thus not a problem. This project, however, has ^{15}N-enriched samples and so the contamination is noticeable and must be addressed. The machine's precision also decreases as the samples get more enriched, adding an uncertainty on top of the growing contamination error.

A first step in minimizing the contamination error is by running the samples in batches in order of enrichment, ranging from my reference samples with natural abundance and my biggest seedlings, to the smallest most enriched samples. By running a few samples at the end of each batch with a known δ^{15}N (called standards, in this case wheat, pine needle and corn) and comparing the measured value with the standards, the sum error of contamination and decreasing precision can be roughly measured.

To counter this error, I decided to round down δ^{15}N to a whole number based on the average error of the batch. The new values are either the same or lower than measured, meaning the new mean for each batch will be lower. The reduction in δ^{15}N values is smaller than the observed differences between the experiment's treatments, ensuring the validity of treatment comparisons.



Level	Number	Mean
Control	78	722,740
Cut	76	630,207
Excluded	79	180,651

Level	Number	Mean
Control	78	661,540
Cut	78	533,233
Excluded	80	183,181

Figure S4. Root and needle delta N for each treatment. The difference between treatments is greater than the contamination error + machines decreasing precision.

Plots showcasing the DNA outliers

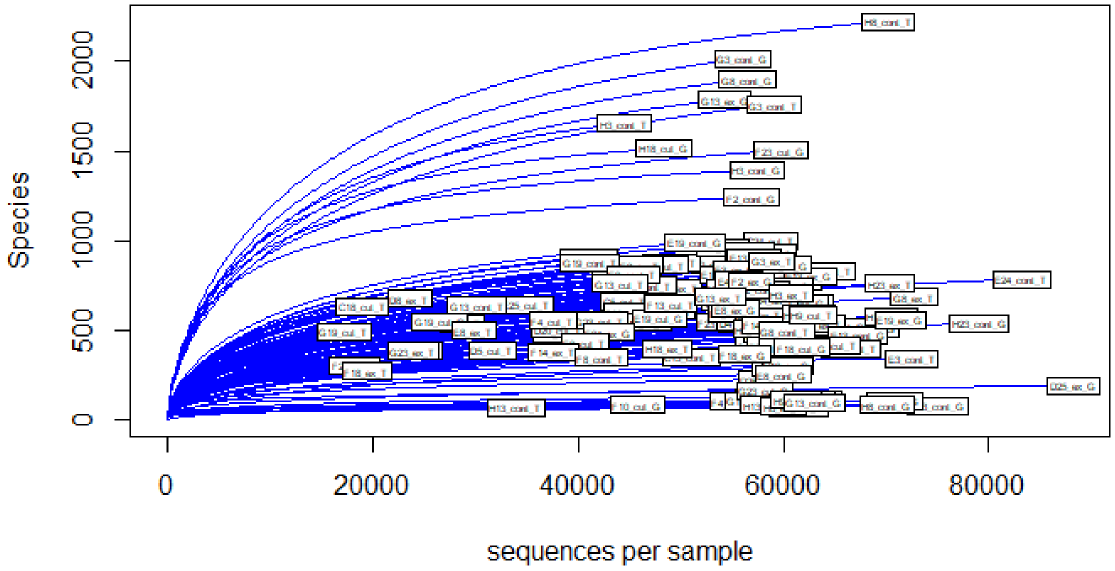


Figure S5. Rarefaction curve (number of OTUs found per number of sequence loops). The outliers are the 10 samples at the top.

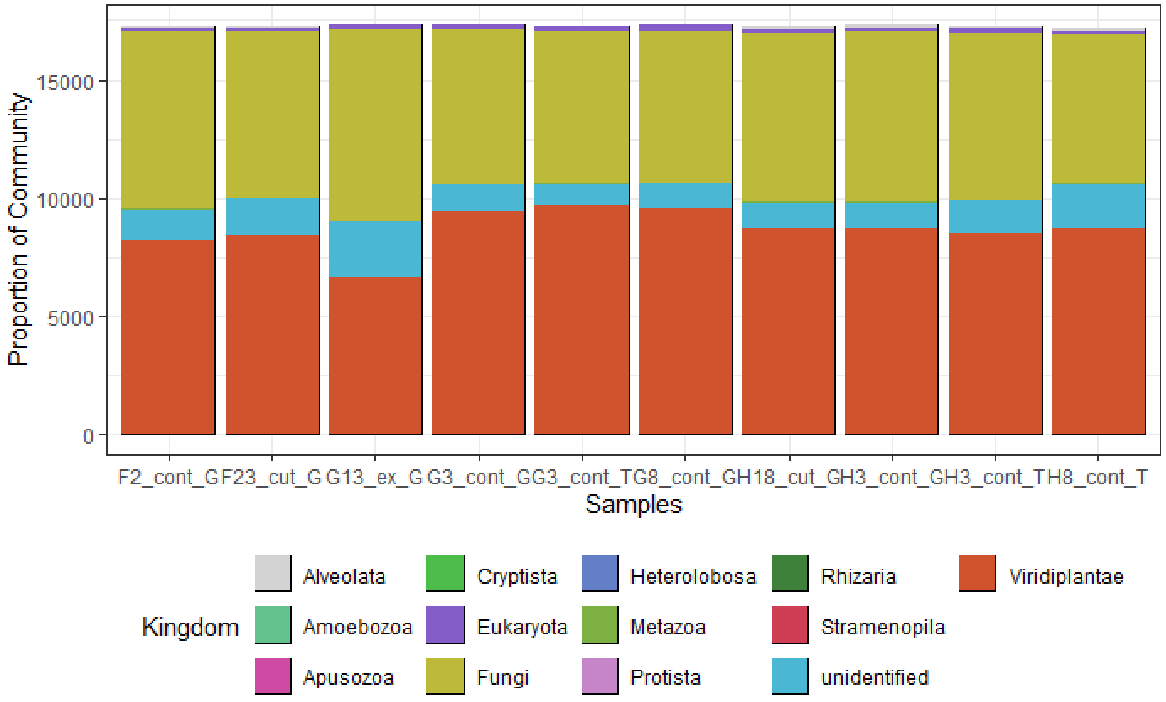


Figure S6. Kingdoms relative abundance of the outliers. Instead of viridiplantea taking up a small part, it now takes up around half of the OTUs.

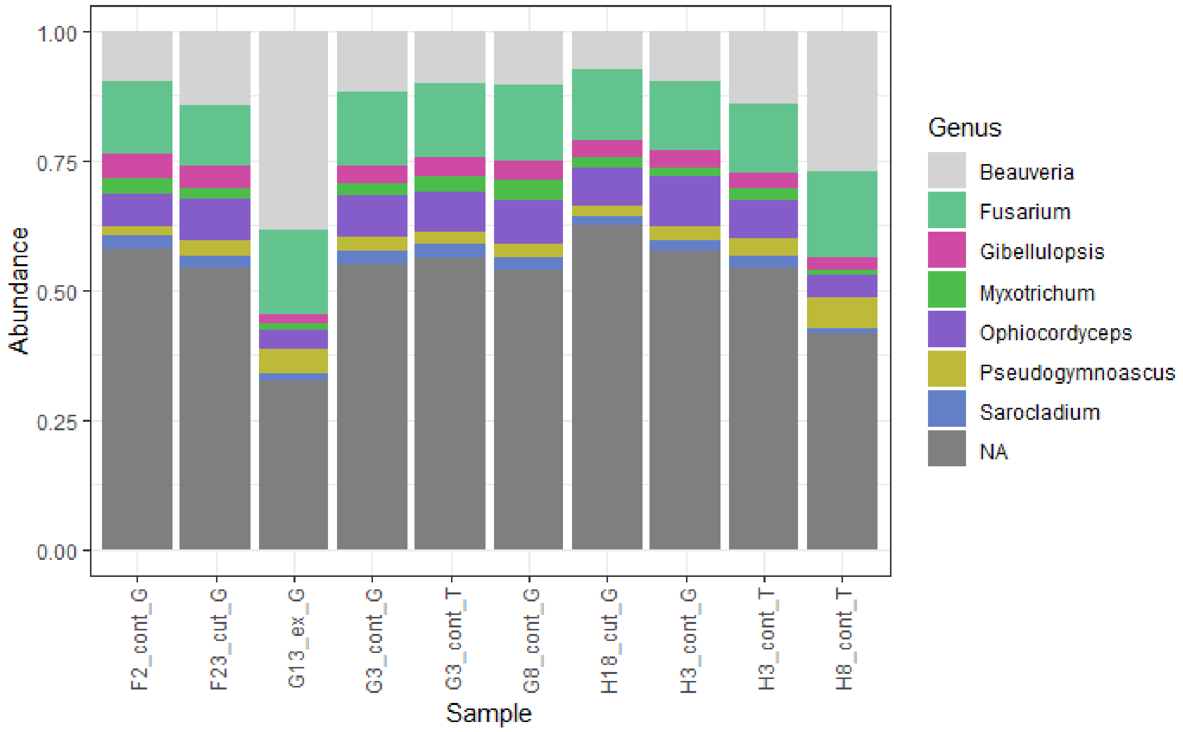


Figure S7. The outliers top 20 OTUs and their relative genus abundance. The genera are very similar to each other and are completely different than the remaining.

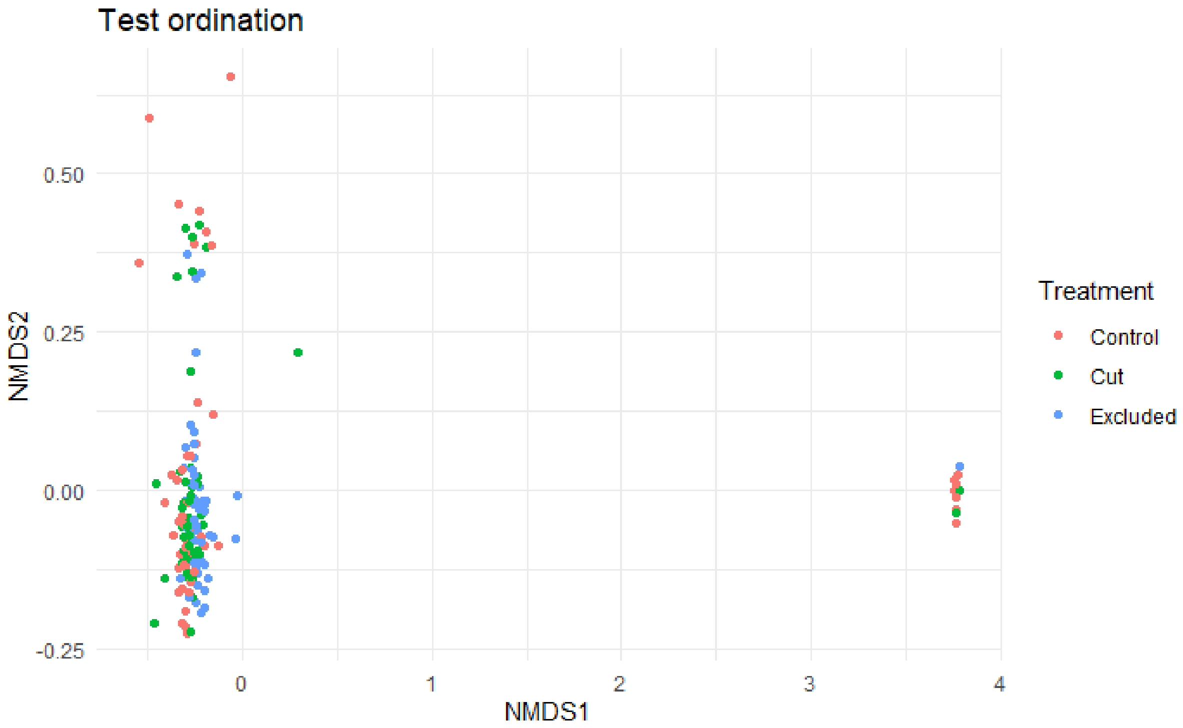
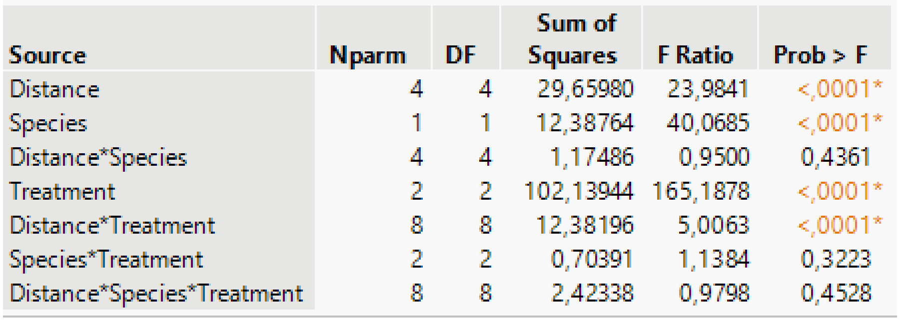


Figure S8. NMDS of only fungal OTUs with the outliers included. The outliers are the 10 dots on the far right.

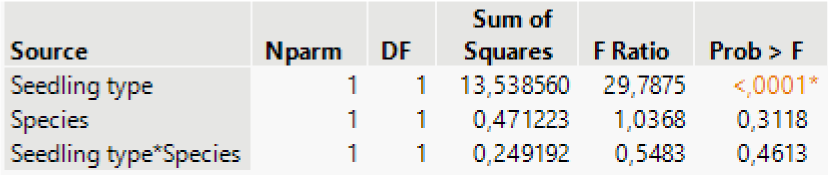
Models for DW and N

*Table S1. Model for DW: log(total DW) = Distance * Species * Treatment . R^2= 71,4% with 237 observations.*



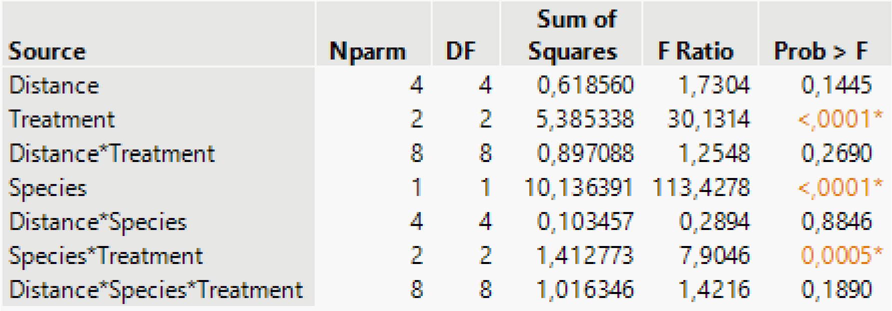
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Distance	4	4	29,65980	23,9841	<,0001*
Species	1	1	12,38764	40,0685	<,0001*
Distance*Species	4	4	1,17486	0,9500	0,4361
Treatment	2	2	102,13944	165,1878	<,0001*
Distance*Treatment	8	8	12,38196	5,0063	<,0001*
Species*Treatment	2	2	0,70391	1,1384	0,3223
Distance*Species*Treatment	8	8	2,42338	0,9798	0,4528

*Table S2. Model for DW release effect: log(DW release effect) = Seedling type * Species. $R^2$of 29 % with 80 observations.*



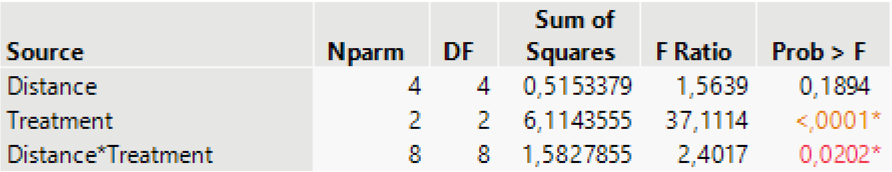
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Seedling type	1	1	13,538560	29,7875	<,0001*
Species	1	1	0,471223	1,0368	0,3118
Seedling type*Species	1	1	0,249192	0,5483	0,4613

*Table S3. Model for biomass allocation: log(root/shoot) = Distance * Species * Treatment. $R^2$of 51,6 % with 237 observations.*



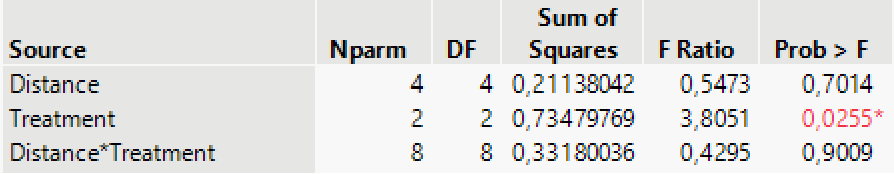
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Distance	4	4	0,618560	1,7304	0,1445
Treatment	2	2	5,385338	30,1314	<,0001*
Distance*Treatment	8	8	0,897088	1,2548	0,2690
Species	1	1	10,136391	113,4278	<,0001*
Distance*Species	4	4	0,103457	0,2894	0,8846
Species*Treatment	2	2	1,412773	7,9046	0,0005*
Distance*Species*Treatment	8	8	1,016346	1,4216	0,1890

*Table S4. Model for biomass allocation with only pine seedlings: log(root/shoot) = Distance * Treatment. $R^2$of 48,7 % with 120 observations.*



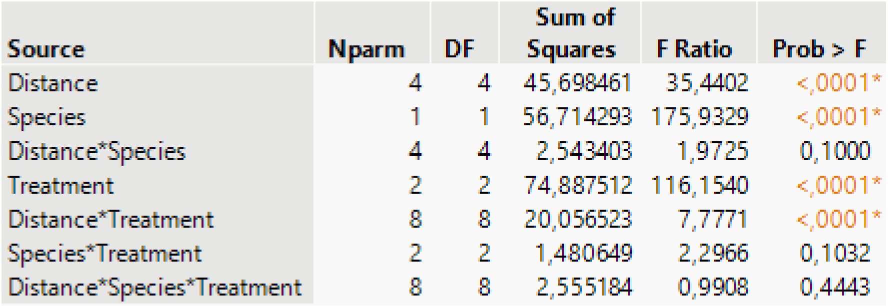
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Distance	4	4	0,5153379	1,5639	0,1894
Treatment	2	2	6,1143555	37,1114	<,0001*
Distance*Treatment	8	8	1,5827855	2,4017	0,0202*

*Table S5. Model for biomass allocation with only spruce seedlings: log(root/shoot) = Distance * Treatment. $R^2$of 11,8 % with 117 observations.*



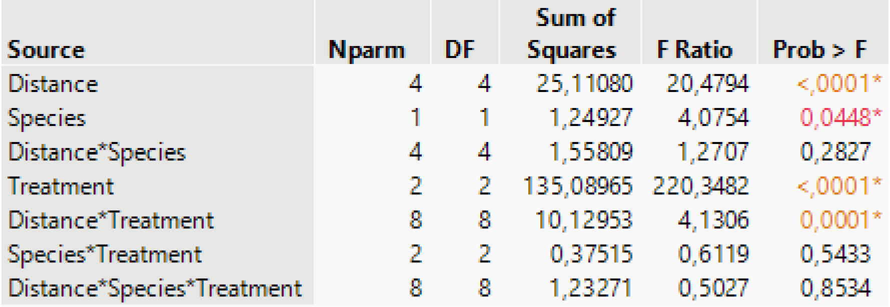
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Distance	4	4	0,21138042	0,5473	0,7014
Treatment	2	2	0,73479769	3,8051	0,0255*
Distance*Treatment	8	8	0,33180036	0,4295	0,9009

*Table S6. Model for total mg N in the needles: log(needle total mg N) = Distance * Species * Treatment.* R^2 *of 75,4 % with 236 observations.*



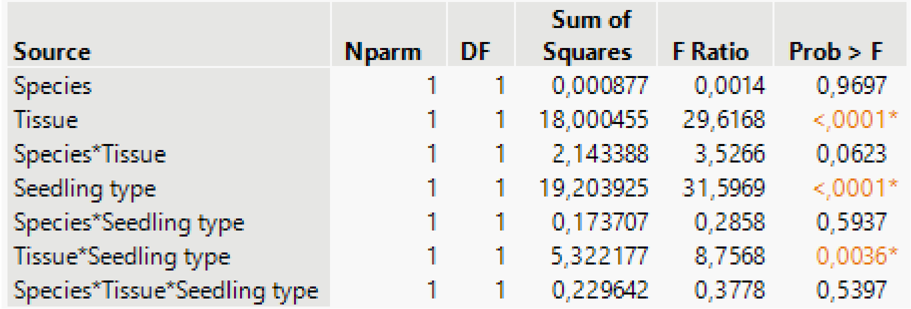
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Distance	4	4	45,698461	35,4402	<,0001*
Species	1	1	56,714293	175,9329	<,0001*
Distance*Species	4	4	2,543403	1,9725	0,1000
Treatment	2	2	74,887512	116,1540	<,0001*
Distance*Treatment	8	8	20,056523	7,7771	<,0001*
Species*Treatment	2	2	1,480649	2,2966	0,1032
Distance*Species*Treatment	8	8	2,555184	0,9908	0,4443

*Table S7. Model for total mg N in the roots: log(root total mg N) = Distance * Species * Treatment.* R^2 *of 73,5 % with 233 observations.*



Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Distance	4	4	25,11080	20,4794	<,0001*
Species	1	1	1,24927	4,0754	0,0448*
Distance*Species	4	4	1,55809	1,2707	0,2827
Treatment	2	2	135,08965	220,3482	<,0001*
Distance*Treatment	8	8	10,12953	4,1306	0,0001*
Species*Treatment	2	2	0,37515	0,6119	0,5433
Distance*Species*Treatment	8	8	1,23271	0,5027	0,8534

*Table S8. Model for N release effect: log(N release effect) = Species * Seedling type * Tissue.* R^2 *of 31,4 % with 157 observations.*



Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Species	1	1	0,000877	0,0014	0,9697
Tissue	1	1	18,000455	29,6168	<,0001*
Species*Tissue	1	1	2,143388	3,5266	0,0623
Seedling type	1	1	19,203925	31,5969	<,0001*
Species*Seedling type	1	1	0,173707	0,2858	0,5937
Tissue*Seedling type	1	1	5,322177	8,7568	0,0036*
Species*Tissue*Seedling type	1	1	0,229642	0,3778	0,5397

Biomass and N data

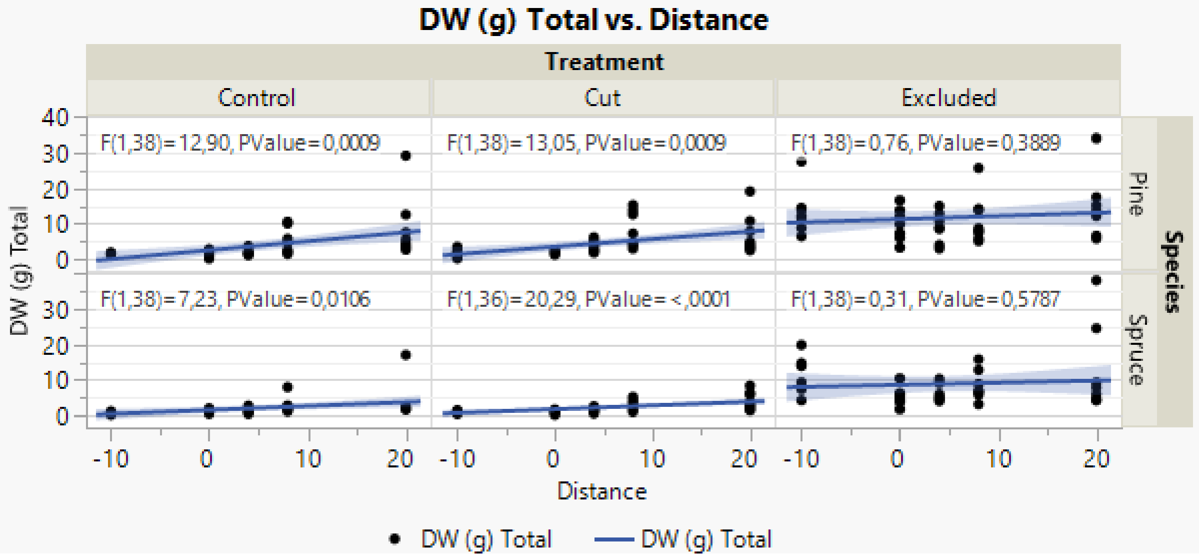


Figure S9. DW vs distance as a continuous variable.

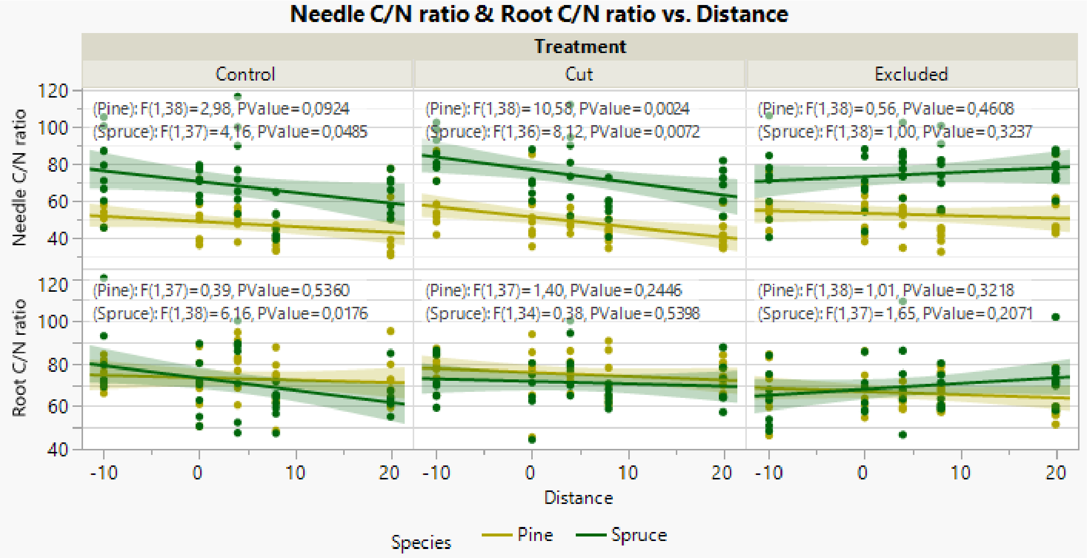


Figure S10. C/N ratio vs Distance.

DNA data

Table S9. Number of OTUs at various stages.

Stage	Number of OTUs
All reads	13457
All reads after removing the 10 outliers	13457
Only fungal reads	8269
Only fungal reads without any unknown genera	3564
Only ECM reads	386

Table S10. Number of OTUs per genera. At least half of all genera only had 1 OTU. At least 75% of all genera has three or less OTUs. On average, each genus has 5,6 OTUs. Meaning there are few genera which have a lot of OTUs, while most only have three or less.

Min	1st quartile	Median	Mean	3rd quartile	Max
1	1	1	5,66	3	347

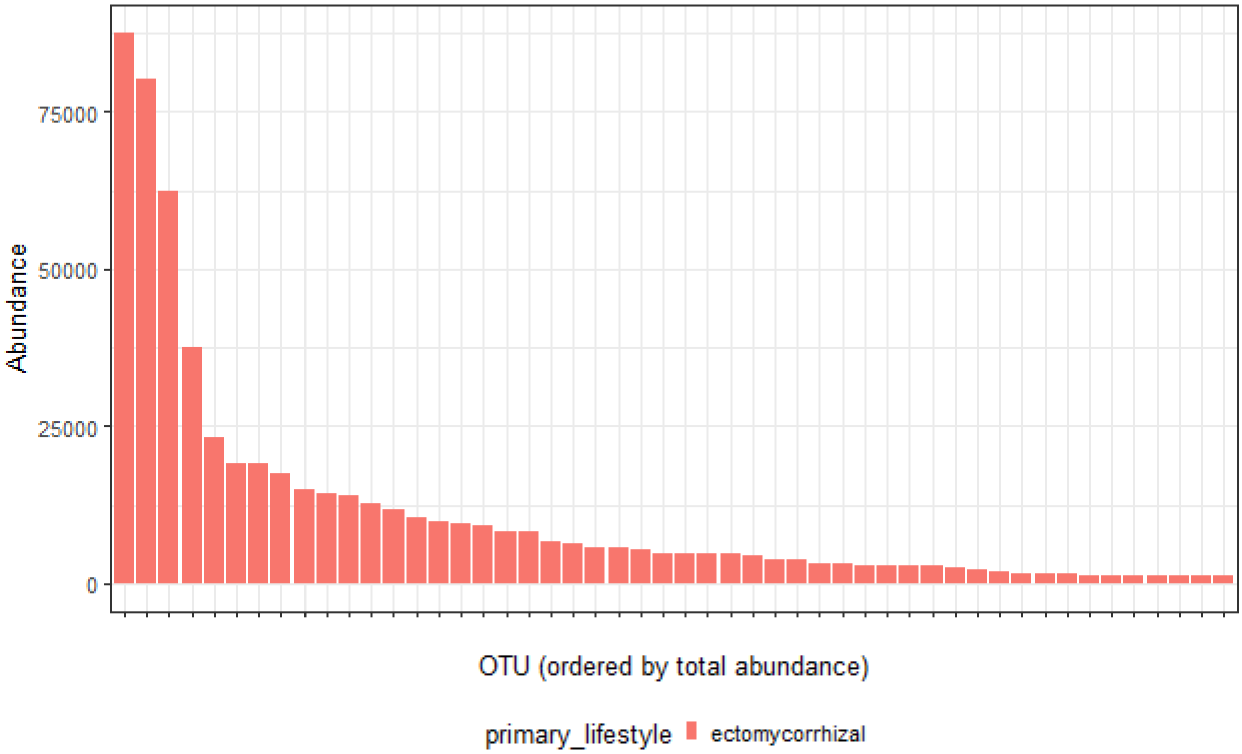


Figure S11. Top 50 ECM OTUs abundance.

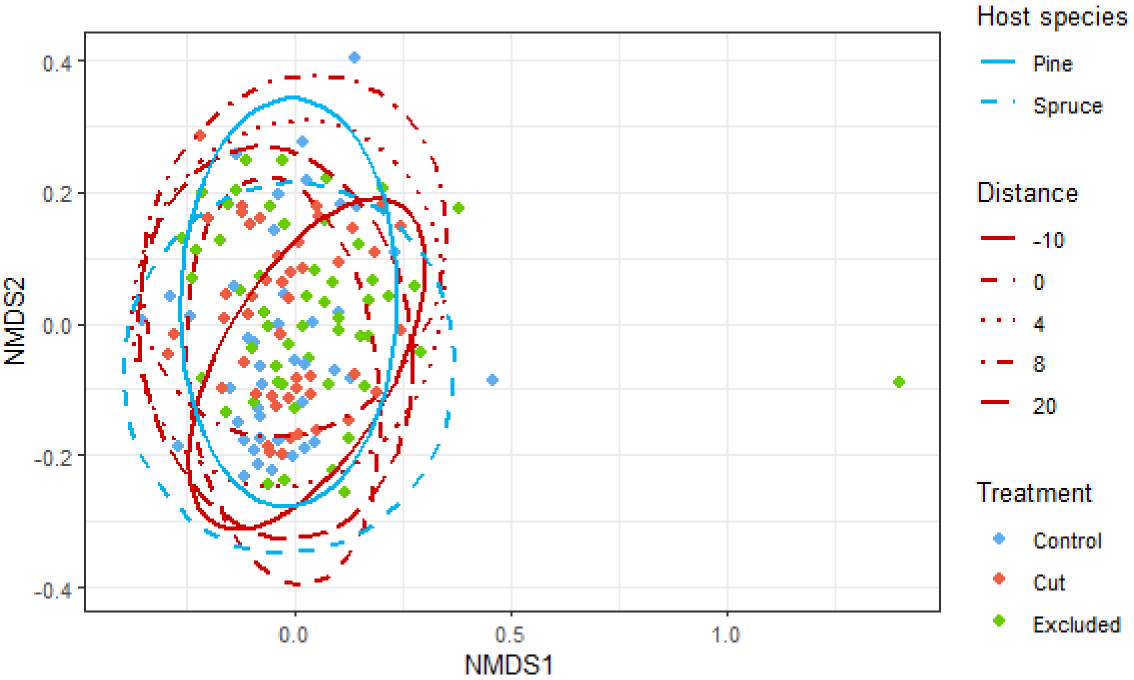
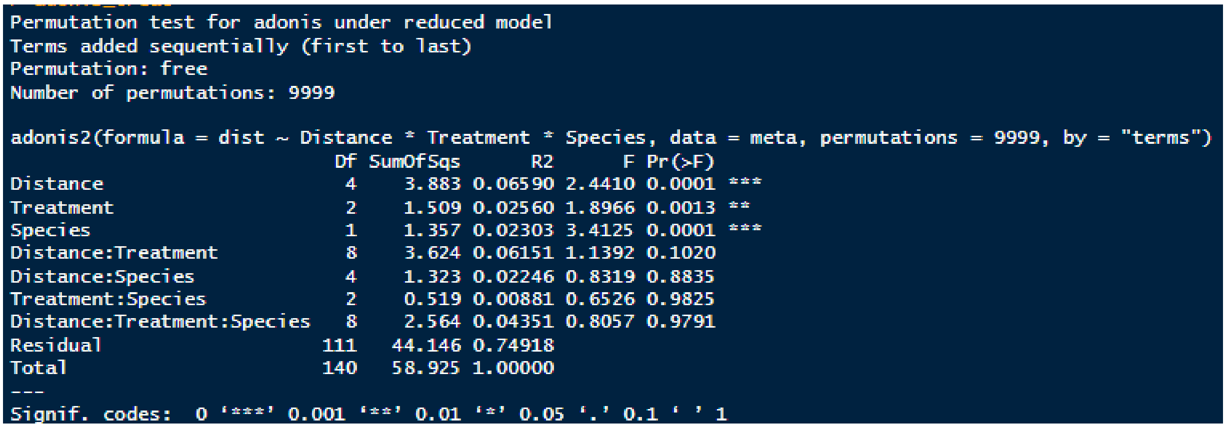


Figure S12. NMDS of only ECM OTUs colored by treatment and circled by distance (red) and host species (blue).

Table S11. PERMANOVA of ECM community composition. Full factorial with distance, treatment, and species with 9999 permutations.



```
Permutation test for adonis under reduced model
Terms added sequentially (first to last)
Permutation: free
Number of permutations: 9999

adonis2(formula = dist ~ Distance * Treatment * Species, data = meta, permutations = 9999, by = "terms")
                           Df SumOfSqs      R2      F Pr(>F)    
Distance                    4    3.883 0.06590 2.4410 0.0001 ***
Treatment                   2    1.509 0.02560 1.8966 0.0013 ** 
Species                     1    1.357 0.02303 3.4125 0.0001 ***
Distance:Treatment          8    3.624 0.06151 1.1392 0.1020    
Distance:Species            4    1.323 0.02246 0.8319 0.8835    
Treatment:Species           2    0.519 0.00881 0.6526 0.9825    
Distance:Treatment:Species  8    2.564 0.04351 0.8057 0.9791    
Residual                  111   44.146 0.74918                  
Total                     140   58.925 1.00000                  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table S12. Results from DW regression on diversity parameters showing no significant p-values and low goodness-of-fit values.

Metric	**Model**	**Slope (±SE)**	**Test z/tz/t**	**p-value**	**Goodness-of-fit**
Richness	Negative-binomial GLM	0,00811 ± 0,00792	z = 1,025	0,305	θ = 3.15; AIC = 1109.4
Shannon	Linear regression (Gaussian)	0,00477 ± 0,00719	t = 0,664	0,508	R^2 = 0,00316

Simpson	Linear regression (Gaussian)	−0,000994 ± 0,00285	t = −0,348	0,728	R² = 0,00087
Evenness	Linear regression (Gaussian)	0,00159 ± 0,00219	t = 0,724	0,470	R² = 0,00379

Soil moisture content

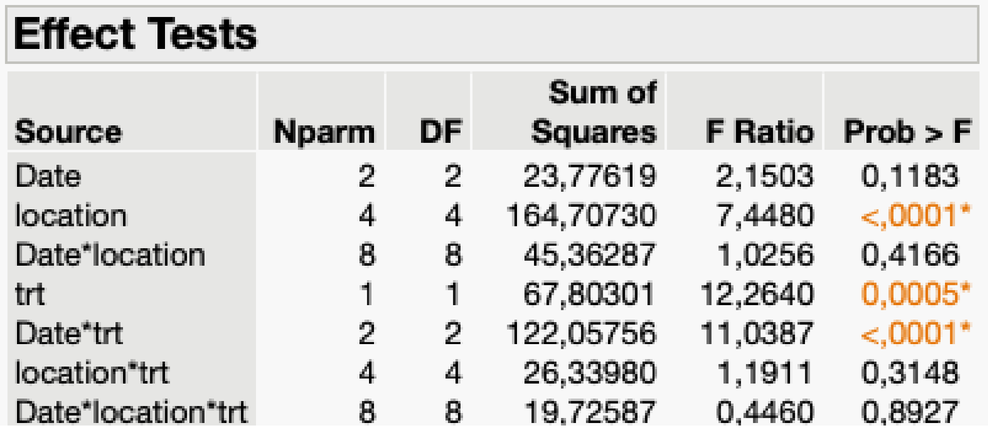
The soil moisture content was measured on three occasions during the summer of 2022 (two years prior harvesting the seedlings for this thesis) with a handheld TDR probe:

June 28 (2022): 160 observations (Only controls and exclusion treatments)

July 7th (2022): 80 observations (Only controls and exclusion treatments)

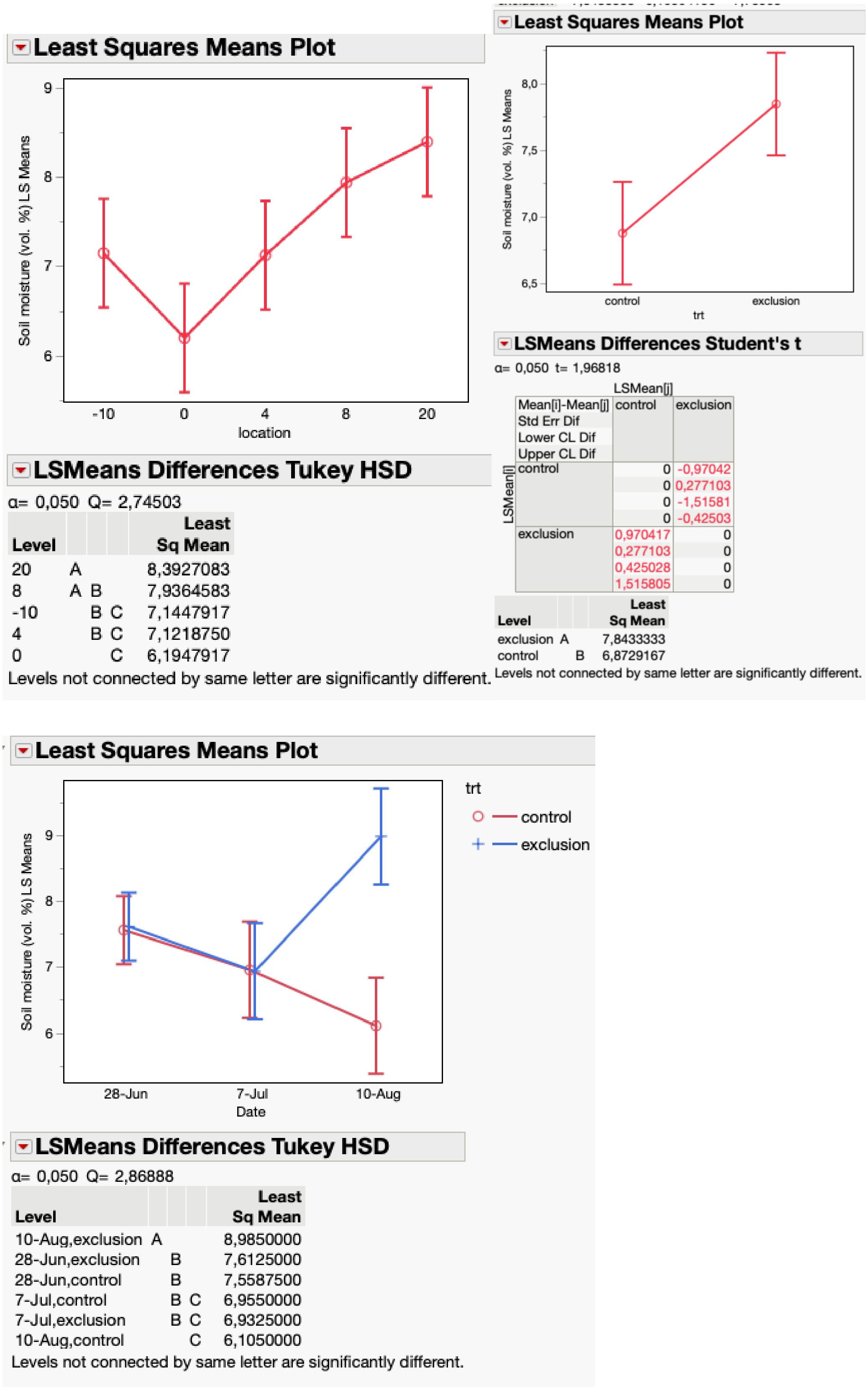
Aug 10th (2022): 120 observations (all three treatments, control, cut, and exclusion). Because the cut treatment was missing from two measurement occasions, only the control and excluded treatment are included in the analysis. This means that N=80+80 on June 28, and N=40+40 in July and August.

Table S13. Standard least squares model of moisture content with full factorial of date of measurement (date), distance to the forest edge (location), and treatment (trt).



Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Date	2	2	23,77619	2,1503	0,1183
location	4	4	164,70730	7,4480	<,0001*
Date*location	8	8	45,36287	1,0256	0,4166
trt	1	1	67,80301	12,2640	0,0005*
Date*trt	2	2	122,05756	11,0387	<,0001*
location*trt	4	4	26,33980	1,1911	0,3148
Date*location*trt	8	8	19,72587	0,4460	0,8927



LSMeans Differences Tukey HSD

α= 0,050 Q= 2,74503

Level				Least Sq Mean
20	A			8,3927083
8	A	B		7,9364583
-10		B	C	7,1447917
4		B	C	7,1218750
0			C	6,1947917

Levels not connected by same letter are significantly different.

LSMeans Differences Student's t

α= 0,050 t= 1,96818

		LSMean[j]	
	Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	control	exclusion
LSMean[i]	control	0 0 0 0	-0,97042 0,277103 -1,51581 -0,42503
	exclusion	0,970417 0,277103 0,425028 1,515805	0 0 0 0

Level			Least Sq Mean
exclusion	A		7,8433333
control		B	6,8729167

Levels not connected by same letter are significantly different.

LSMeans Differences Tukey HSD

α= 0,050 Q= 2,86888

Level				Least Sq Mean
10-Aug,exclusion	A			8,9850000
28-Jun,exclusion		B		7,6125000
28-Jun,control		B		7,5587500
7-Jul,control		B	C	6,9550000
7-Jul,exclusion		B	C	6,9325000
10-Aug,control			C	6,1050000

Levels not connected by same letter are significantly different.

Figure S13. Plots and significance letters for soil moisture. Distance gradient (a), comparison between control and in the bucket (b) and across different measurement dates (c).

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:

☒ YES, I 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 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.

SENASTE UTGIVNA NUMMER

2024:06	Författare: Emil Gustavsson Demand-driven fertilization (BAG) effects on stream water chemistry. A study in the Undersvik High-yield Experimental Forest.
2024:07	Författare: Per Schönning Comparing living and dead tree carbon stocks between burnt and managed forests – A chronosequence analysis between burnt and managed forests
2024:08	Författare: Marcus Björs Gap-edge Effects on Seedling Growth, N Uptake and Associated Ectomycorrhizal Fungi
2024:09	Författare: Carlos Miguel Amante Aragon Effects of Forest Management on Understory Plant Traits and Biodiversity - A chronological analysis of fire burned and managed forests
2024:10	Författare: Joseph Olugbenga Kayode Improved logging in tropical rainforest for carbon sequestration – A case study in Borneo, Malaysia
2024:11	Författare: Millie Johnson Disentangling the Effects of Goose Disturbance and Warming on Aboveground and Belowground Processes: Insights from the Thawing High Arctic Tundra of Svalbard
2024:12	Författare: Ayhan Yener Measurement of water content in green stems using thermal properties
2024:13	Författare: Jonas Laurens Kappen Tree species mixture under drought stress: results of results of Quercus gambelii – Pinus ponderosa stands in the Southwestern US
2024:14	Författare: Dorothea Milena Jentzsch The change of microclimate in riparian buffers following clearcutting of adjacent forest stands.
2024:15	Författare: Gayan Sanjeewa Samarasighe The impact of climate change and drainage maintenance strategies on boreal peatlands water storage capacity
2025:01	Författare: Hanna Anundi Skogsbad i cancerrehabilitering? En utvärdering av en naturbaserad intervention i cancervården.
2025:02	Författare: Oscar Andersson Warming alters root trait strategies and enchances overyielding in boreal tree seedling mixtures
2025:03	Författare: Julian Säflund Forest edge effect on seedling growth, C/N allocation and fungal associations. A closer look at 3-year-old seedling growth patterns above- and belowground and their mycorrhizal symbionts according to their distance to a forest edge.