Establishment success of *Pinus sylvestris* seedlings with fertilization and mechanical site preparation: survival, growth and ectomycorrhizal fungal responses

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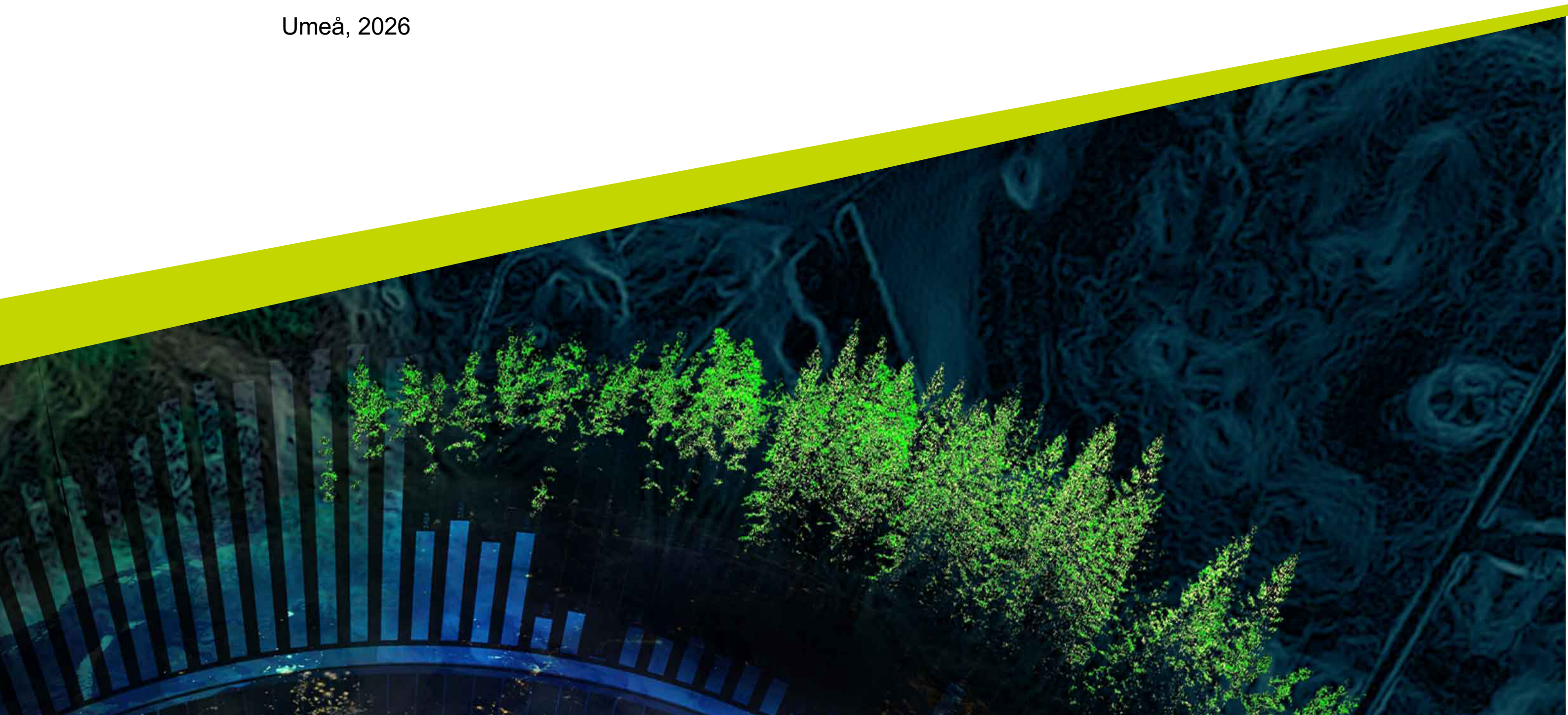
Degree project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Forest Ecology and Management
Masters's of Forest Ecology and Sustainable Management
Examensarbeten / SLU, Institutionen för skogens ekologi och skötsel
2026:09 • ISSN 1654-1898
Umeå, 2026



Establishment success of *Pinus silvestris* seedlings with fertilization and mechanical site preparation: survival, growth and ectomycorrhizal fungal responses

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Credits:	30 credits
Level:	A2E
Course title:	Master’s of Forest Ecology and Sustainable Management
Course code:	EX1040
Programme/education:	Forest Ecology and Sustainable Management
Course coordinating dept:	Forest Ecology and Management
Place of publication:	Umeå
Year of publication:	2026
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:	2026:09
ISSN:	1654-1898
Keywords:	Boreal reforestation, ectomycorrhizal fungi, ITS metabarcoding, mechanical site preparation, organic nitrogen, *Pinus sylvestris*, seedling survival

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Abstract

Successful establishment of conifer seedlings is critical for reforestation, yet the effects of fertilization and mechanical site preparation on early seedling performance remain poorly understood. This field study investigates the effects of Silvivo, an organic nitrogen-based fertilizer treatment, on *Pinus sylvestris* seedlings over one growing season in northern Sweden. Seedlings were planted in June and harvested in October across four treatment groups — one control and three treatment combinations incorporating Silvivo and mechanical site preparation. Survival, total biomass, root collar diameter, and root-to-shoot ratio were measured to assess growth and establishment. Ectomycorrhizal (ECM) fungal community composition was characterized using ITS metabarcoding.

The Silvivo amendment increased total biomass and root collar diameter across treatments, and improved seedling survival in plots without mechanical soil preparation. Root-to-shoot ratio was only marginally affected when seedlings were grown in mounded soil. There was shift in ECM fungal community composition in response to the fertilizer treatment, but this shift was not significant. The observed growth and survival responses, despite the limited ECM community responses, suggests that the changes in the ECM community may not be the primary driver of the seedling response to Silvivo during the first growing season. These findings indicate that organic nitrogen fertilization may improve early establishment metrics in *Pinus sylvestris*, particularly when mechanical site preparation is not utilized, and highlight the importance of site preparation method when evaluating fertilizer efficacy in boreal reforestation.

Keywords: Boreal reforestation, ectomycorrhizal fungi, ITS metabarcoding, mechanical site preparation, organic nitrogen, *Pinus sylvestris*, seedling establishment

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Abbreviations

Abbreviation	**Description**
C	Carbon
CRF	Controlled-release fertilizer
ECM	Ectomycorrhizal Fungi
ITS	Internal Transcriber Spacer
MSP	Mechanical Soil Preparation
N	Nitrogen
RCD	Root Collar Diameter
R:S ratio	Root-to-Shoot Ratio
SOM	Soil Organic Matter

1. Introduction

1.1 Importance of forestry in Sweden

The forestry sector in Sweden is incredibly large. Forests cover 70% of the land in Sweden (Swedish Forest Industries 2026), and an average of 231,000 hectares of forest were felled annually from 2019 to 2023 (Skogsstyrelsen 2024). It employs approximately 140,000 individuals, and in 2024, exported goods were valued at 185 billion SEK (Swedish Forest Industries 2026).

1.2 Reforestation challenges in boreal forests

Reforestation represents a major investment in Swedish forestry and is essential for maintaining future forest productivity. According to the Swedish Forestry Act, sufficient regeneration must be established within three years of harvest (Skogsstyrelsen 2025). To accomplish this, each year approximately 1.5 billion SEK is spent to plant 400 million seedlings (Skogsstyrelsen 2024; Swedish Forest Industries 2026). The majority of these seedlings are *Pinus sylvestris* L. (Scots pine) and *Picea abies* (L.) H. Karst (Norway spruce), which together account for over 90% of planted seedlings (Skogsstyrelsen 2024). During 2023-2024, approximately 50-60% of seedlings showed high vitality after one growing season, and approximately 20% of seedlings died (Öhlund et al. 2025). Given the substantial resources invested in reforestation, improving regeneration success is essential for reducing seedling losses and supporting long term forest productivity.

1.2.1 Mechanical site preparation

Mechanical site preparation (MSP) has been a standard forest management practice for decades. Scarification breaks up the thick humus layer and litter while exposing the mineral soil (Nyland et al. 2016) which is considered essential for seedlings to establish and outcompete vegetation (Grossnickle 2005; Nilsson et al. 2010; Nyland et al. 2016). In 2024, Skogforsk published a report of forest regeneration statistics that surveyed more than 460 forest stands across Sweden, and which found over 90% of stands implemented MSP prior to regeneration (Öhlund et al. 2025), however the practice still faces criticism.

Reasons include damaging archaeological remains and erosion in dry sites (Löf et al. 2012), and destruction of winter grazing pastures critical for reindeer husbandry (Kivinen et al. 2010; Häggström et al. 2021). It is understood that there are multiple MSP techniques and such negatives are not uniformly associated with all of them (Löf et al. 2012; Häggström et al. 2021). For instance, mounding is a technique which creates isolated points of overturned mineral soil while maintaining the integrity of the surrounding humus layer (Häggström et al. 2021). Nonetheless, this has sparked debate and discussion about MSP in Sweden, even to the point that both individuals and entire organizations are exploring alternative management options. In addition, there are areas where MSP may not be appropriate or feasible, due to a high water table or obstacles like boulders and deadwood (Öhlund et al. 2025).

1.2.2 Nitrogen limitation in boreal forests

In the boreal forest, plant growth is predominantly constrained by nitrogen (N) availability, a limitation widely attributed to the slow rate at which organic N is released from soil through mineralization (Näsholm et al. 1998). Limited nutrient availability reduces the growth rate and establishment success of newly planted seedlings (Castro et al. 2021). More nutrients can increase seedling growth and root development which may subsequently improve the seedling's ability to tolerate stressful environmental conditions by increasing access to soil water (Grossnickle 2005).

However, fertilization in the field comes with challenges. Added nutrients are not always directly taken up by the target plant. Some estimates suggest that plants only utilize 30-40% of the total nutrients released from fertilizers, while the remainder can enter and degrade the environment through N leaching, ammonia volatilization and eutrophication (Negi et al. 2022). To minimize nutrients entering the surrounding ecosystem, control-release fertilizers (CRF) utilize a coating to reduce the rate of nutrient release (Negi et al. 2022; Isakov et al. 2024).

1.2.3 Control-release fertilizers and their effectiveness

A vast array of compounds and materials have been tested in CRF coatings, since effectiveness is determined by the interaction of environmental factors (i.e. temperature, pH and microbial activity) and manufactured traits such as size, shape, coating thickness and material (Negi et al. 2022). Both organic and inorganic coatings have been shown to affect the rate of nutrient release (Negi et al. 2022). These coatings must resist physical wear and remain water-insoluble to perform effectively. Many coating materials have been tested but have associated drawbacks. Sulphur compounds are easily damaged while mineral compounds can dissolve in water (Negi et al. 2022; Isakov et al. 2024) which can result in nutrient dumping. Coatings containing sulphur have also shown to raise the soil pH (Negi et al. 2022). In addition, manufacturing CRF is often more expensive compared to conventional fertilizers.

1.3 Plastic-based coatings and European Union microplastic regulations

CRF coatings must be durable and insoluble in water to effectively avoid nutrient dumping while maintaining a consistent and reliable rate of nutrient release (Isakov et al. 2024). Plastics in the coatings are effective in this regard but persist in the soil and water environments for many years (Isakov et al. 2024), which has increased the concern regarding soil health (Bläsing & Amelung 2018; Isakov et al. 2024). To reduce plastics entering the environment, the European Union has passed legislation to prohibit plastics smaller than 5 mm in many commercial products, which will also apply to fertilizers by October of 2028 (European Commission 2023).

1.4 Plastic free alternatives and Silvivo

This legislation has contributed to growing interest in CRF coatings that do not utilize plastic (Isakov et al. 2024). One such product is Silvivo, developed by Cropcision AB. Silvivo is an amino-acid rich granular fertilizer that is applied directly to seedling roots without causing salt stress. Its nutrient source is derived from yeast extract and intercalated between layers of clay that control release based on soil moisture and temperature (Silvivo 2026).

1.5 Biomass allocation

1.5.1 Why biomass allocation is relevant to newly planted seedlings

When a seedling is transplanted from the nursery into the ground, it is at the most vulnerable stage in its life. In fact, across all of Sweden, 20% of planted seedlings die during the first three years (Öhlund et al. 2025). While there are multiple biotic and abiotic factors affecting seedling survival, including simply being eaten (Häggström et al. 2021), the cause of mortality is often rooted in environmental stress, namely related to water and nutrients (Grossnickle 2005; Nilsson et al. 2019). Therefore, it is in the seedling's best interest to grow quickly and as much as possible during the first few seasons in the field. But is all growth equally important to improving the chances of survival? There are limited resources in the boreal forests and dedicating them to certain parts of the seedling may increase odds of surviving, specifically, the roots.

At the time of transplanting the roots are at their smallest and have limited access to nutrients since the root are directly responsible for acquiring water for transpiration and nutrients for metabolic processes and growth (Grossnickle 2005; Nilsson et al. 2010). Therefore, seedlings which develop larger and more robust root systems are favorable to seedlings with a smaller root system (Burdett et al. 1983). Grossnickle (2005) estimates poor root development reduces survival to merely 35%. The goal of many silvicultural tools (i.e. MSP, nutrient supplementation, planting season, nursery germination and genetic optimization) is to maximize healthy seedlings and regeneration success (Burdett et al. 1983; Grossnickle 2005; Nilsson et al. 2010).

1.5.2 What causes the shift in biomass allocation?

It may be of interest in improving reforestation and reducing wasted resources, labor and money by supporting root development which increases the odds of seedling survival. One method of achieving this is N addition. Supplementing N from organic sources, such as amino acids, typically causes the seedling to allocate more resources to root development than shoot development, while inorganic N supplements typically favor the opposite (Gruffman et al. 2013).

Plants can utilize amino acid N more efficiently than inorganic forms of N, like nitrate or ammonium, as it is already in a reduced chemical state that requires less metabolic processing (Gruffman et al. 2013). Amino acid N is heavily retained in the roots even when the plant's carbohydrate supply is limited (Gruffman et al. 2012). There are a few possible mechanisms behind this observation: Compared to inorganic N, amino acid N may bind tightly to soil particles, stimulating more root development. In addition, amino acid N can provide carbon (C) that may further support root development (Gruffman et al. 2012).

1.6 Ectomycorrhizal fungi interactions with seedlings

While an important metric, the root biomass is not the only factor which affects nutrient uptake. The soil fungal community is an integral part of a functioning ecosystem, especially in the context of boreal forests. As addressed previously, a typical characteristic of boreal forests is that plant growth is limited by N availability. Fungi are fundamental for plant nutrient acquisition, whether through interactions with decaying organic matter or direct symbiotic relationships with living plants (Read & Perez-Moreno 2003) and can also enhance the host root's water acquisition by increasing the absorptive surface area and improving hydraulic conductivity at the root-soil interface (Brunner et al. 2015).

The form of available N shapes both the ectomycorrhizal (ECM) fungal community composition and root development since the variation between ECM genera differ in their nutrient acquisition and tolerance to different N forms. LeDuc et al. (2013) found that the abundance of *Amanita*, *Cortinarius*, *Russula* and *Tricholoma* was positively correlated with the availability of organic N. Matrinez-Nevárez et al. (2025) describes a similar pattern and showed high levels of inorganic N reduces

ECM colonization. This sensitivity to N form extends to root morphology, as Gruffman et al. (2012) showed that the form of applied N differentially affects both fungal colonization and root architecture. Beyond individual taxa, community-level composition also determines the breadth of acquisition, as particular ECM species occupy distinct seasonal niches that may collectively sustain N cycling across the growing period (Heinonsalo et al. 2015).

ECM fungi may vary considerably in how much C is gained from the host plant. For example, *Cortinarius* can form extensive mycelial networks but require substantial C inputs from the host in return (Anthony et al. 2024). ECM groups capable of surviving on reduced C inputs may therefore hold a competitive advantage, outcompeting more C demanding fungal genera while simultaneously freeing valuable photoassimilates for use elsewhere in the plant (Smith & Read 2008).

These C trade-offs are particularly consequential in seedlings, where small plant size constrains total photosynthesis, C assimilation and glucose production, making every unit of sugar energetically precious. Notably, the uptake of N from amino acid sources is largely independent of carbohydrate stores, unlike the acquisition of inorganic N, which places a greater demand on available C (Gruffman et al. 2013). Access to amino acid N through ECM partnerships could therefore allow seedlings to redirect limited photo-assimilates away from costly uptake of inorganic N and toward other processes, such as growth and metabolism.

There is a complex relationship between tree growth and microbial life, specifically ECM fungi. Mycorrhizal communities are an integral pathway for plants to acquire nutrients and water. There are ECM species which are typically present in boreal forests that can improve plant survival such as *Suillus* (Bending et al. 2002), *Cortinarius*, *Piloderma*, and *Russula* (Lindahl et al. 2021), *Laccaria*, and *Thelephora* (Hilszczańska et al. 2008). However, there has been difficulty in distinguishing if these organisms are the drivers of plant growth or if there is a more complex interaction with abiotic factors (Anthony 2025).

1.7 Metabarcoding for fungi identification

Scientists previously identified fungi based on morphological and growth characteristics, but this lacks precision and consistency. Current practices utilize DNA sequencing to identify fungi. The most common DNA region used is the Internal Transcribed Spacer 2 (ITS 2), found within the ribosomal RNA genes. This region changes quickly between species, making it useful for distinguishing fungal taxa.

Since the ITS region does not code for proteins, it typically experiences less evolutionary pressure which results in lots of variation. It is now considered the standard "barcode" for fungal identification, supported by databases like UNITE. To ensure the accuracy and reliability of sequencing results, Novogene applied quality control measures at each step throughout the process (sample testing, PCR, purification, library preparation, and sequencing) (Novogene 2026).

1.8 Research objectives and hypotheses

The purpose of this study is to evaluate the impact of applying a nutritional supplement directly to the root zone at the time of planting. To determine this, the project will evaluate whether Silvivo impacts the survival and growth of Scots pine seedling after one growing season. We will also evaluate the impact of Silvivo on ECM colonization. Finally, this study will evaluate how mechanical site preparation impacts these potential changes.

Hypotheses

H1: The application of Silvivo will improve the rate of seedling survival.

H2: Seedlings receiving Silvivo will exhibit greater growth than unfertilized seedlings, as well as increase the ratio of belowground biomass to aboveground biomass.

H3: Seedlings treated with Silvivo will exhibit altered colonization of ECM compared to unfertilized seedlings.

H4: Silvivo will improve the metrics of the first three hypotheses regardless of the use of MSP.

2. Materials and Methods

2.1 Experimental location

The trial location was in Avanäs within Västerbotten County (64.1583, 20.21403). The seedlings were planted on 13/05/2025 and harvested on 06/10/2025. The area is moderately fertile black soil with ditching (T21) and during the trial period there was approximately 341 mm of rain (Griddad nederbörd- och temperaturdata 2025).

2.2 Planting material

At the trial location, seven treatment plots were selected by manually evaluating the soil preparation. The seven plots were to have moderate soil moisture and mounds of overturned organic humus capped with exposed mineral soil, all the while being as uniform as possible to replicate the growing microclimate among the plots.

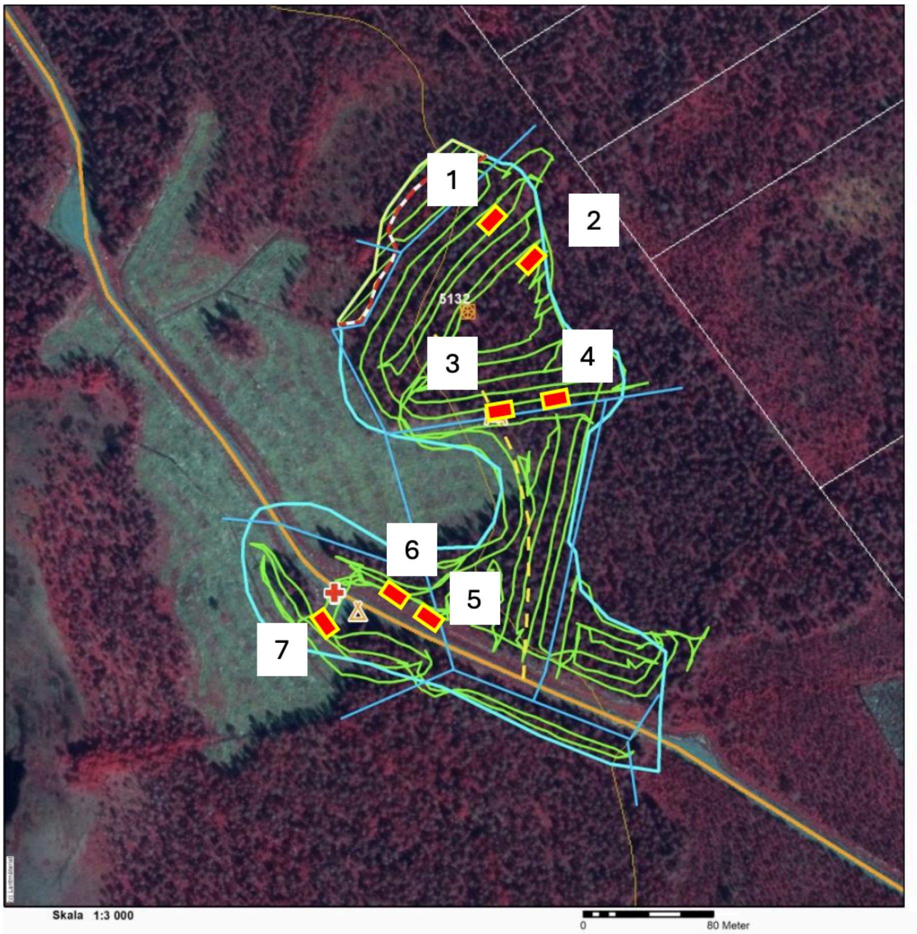

Figure 1. Trial location in Avanäs with marked plots (red retangles). Picture from Cropcision AB.

At each plot, there were three treatments of nutrient supplementation and a negative control. At least five Scots pine seedlings were planted manually into exposed mineral soil at each plot, one per capped mound, using a *planteringsrör*. The respective nutrient supplementation treatment was added into the *planteringsrör* before the seedling to ensure direct contact with the roots and ease of replication. Finally, all planting and treatments were duplicated by planting seedlings directly into the intact organic layer adjacent a planted seedling. See the Figure 2 for a visual representation of the plots.

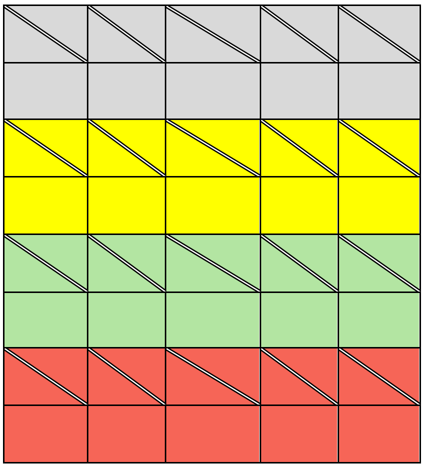

Figure 2. Aid to visualize the layout of individual plots. Control (grey), Silvivo 40 (yellow), Silvivo 80 (green), and Reference (red). A diagonal line represents a seedling planted into mineral soil exposed via MSP. No diagonal line indicates a seedling planted directly into the organic layer of soil. This style of treatment was replicated at all seven plots.

Four variants of nutrient supplementation were implemented to test the hypotheses: Two dosages of Silvivo, which delivered 40 mg N and 80 mg N, along with a positive control, an amino acid monophosphate salt which delivered 40 mg N, and no nutrient supplement. These will be referred to as Silvivo 40, Silvivo 80, Reference and Control, respectively.

Each treatment was applied to a at least 35 Scots pine seedlings, but additional seedlings were also supplemented in the Reference, Silvivo 40 and Silvivo 80 treatments.

2.3 Harvesting

The seedlings were harvested during the first week in October to maximize the time since planting for potential responses while avoiding an overnight freeze. The same protocol was followed at each of the seven trial plots.

All surviving seedlings were visually evaluated based on their physical appearance and three seedlings were selected from each of the four treatments to be harvested.

These chosen seedlings were considered representative of the surviving individuals within each treatment. The seedlings were manually harvested using a shovel. While harvesting, we maintained wide margins to avoid potential damage to the root system. The same harvesting procedure was applied to seedlings planted in both the prepared and unprepared soil.

Once harvested, excess soil was removed from the seedling by gentle shaking. A sample from the soil, which was in close contact with the roots, was collected into a plastic bag, as well as roots which grew outside of the peat plug. These were stored in the same plastic bag as the soil. A separate bag was used for each treatment within each plot. For example, the roots and soil from all three seedlings from 'plot 2-control-unprepared soil' were stored in the same bag. Each bag was immediately stored on dry ice during field work and transport before long-term storage at -20°C.

2.4 Data collection

2.4.1 Survival

During harvest, seedling survival was assessed by counting all the surviving seedlings. The criterium for a seedling to be counted as 'survived' was green needles. If a seedling had no green needles, no needles present or was entirely missing it was counted as 'not survived'.

2.4.2 Biomass

The seedlings were gently washed to remove excess soil and non-seedling organic material, i.e. moss and humus. Once washed, the aboveground biomass was separated by cutting directly where seedling grew out of the peat plug. The field roots which had grown outside the peat plug were also collected to represent the belowground biomass. The aboveground biomass was stored separately from the belowground biomass. All biomass was dried in a 65°C oven to remove as much water as possible. The dry mass of the aboveground and belowground biomasses was recorded separately.

2.4.3 Root Collar Diameter

The root collar was defined as the point of transition between the stem and the root system, located at the top of the peat plug for containerized seedlings. The root collar diameter of the seedlings was measured using digital calipers. To minimize measurement error and mechanical damage to the delicate cambium layer, the caliper jaws were applied with consistent, light pressure until contact was established without compressing the bark.

2.5 Statistical analysis of growth

All categorical analyses were performed using JMP Student Edition (2025). The resulting contingency tables and variance graphs were generated using the same program.

2.5.1 Survival

Since the expected cell counts were low (< 5), Fisher's Exact Test would be used in place of the Pearson's Chi Square test. Post-hoc pairwise comparisons when the Fisher's Test indicated significance.

2.5.2 Biomass

The total biomass was calculated by adding the aboveground and belowground dry biomasses. The root to shoot ratio was measured to determine if the treatments influenced biomass allocation between the root systems and shoots. The belowground biomass was divided by the aboveground biomass to give the root-to-shoot ratio for each seedling.

$$\frac{Belowground\ biomass\ (g)}{Aboveground\ biomass\ (g)} = Root : Shoot$$

Since this data was continuous, normality was assessed prior to variance analysis to ensure that the assumptions of parametric testing were satisfied.

2.5.3 Root Collar Diameter

The null hypothesis stated that there were no significant differences in mean root collar diameter growth in response to the treatments. In the event of a significant F-test ($p < 0.05$), a post-hoc Tukey's Honestly Significant Difference (HSD) test was conducted. This allowed for all pairwise comparisons between the three treatments and the control while controlling for Type I error rate.

2.6 Metagenomic Analysis

Due to financial restrictions only samples from the Control and Silvivo 40 treatments were submitted to Novogene for DNA extraction and sequencing. 5 replicates from the Control and 5 from Silvivo 40 were analyzed. The name distinguishes between fertilization treatment, tissue type and the use of MSP. See Table 1 for specific nomenclature within the Novogene reported results.

Table 1. Abbreviations for treatments represented in metagenomic analysis.

Name	Treatment	Tissue type	MSP
CR1	Control	Root	Prepared

SR1	Silvivo 40	Root	Prepared
CS1	Control	Soil	Prepared
SS1	Silvivo 40	Soil	Prepared
CR2	Control	Root	Unprepared
SR2	Silvivo 40	Root	Unprepared
CS2	Control	Soil	Unprepared
SS2	Silvivo 40	Soil	Unprepared

3. Results

3.1 Survival

3.1.1 Survival with MSP

To evaluate hypothesis 1, which predicts Silvivo increases the survival rate of the seedlings, we counted the living and dead seedlings after one growing season (Figure 3). Since multiple cell counts are less than 5, a Fisher's Exact Test replaces the standard Pearson's Chi-Square Test. There is no significant association (p = 0.090) between the treatments and survival observed in seedlings grown with MSP. Hypothesis 1 was not supported as there is no significant difference in survival among the treatments when MSP is utilized.

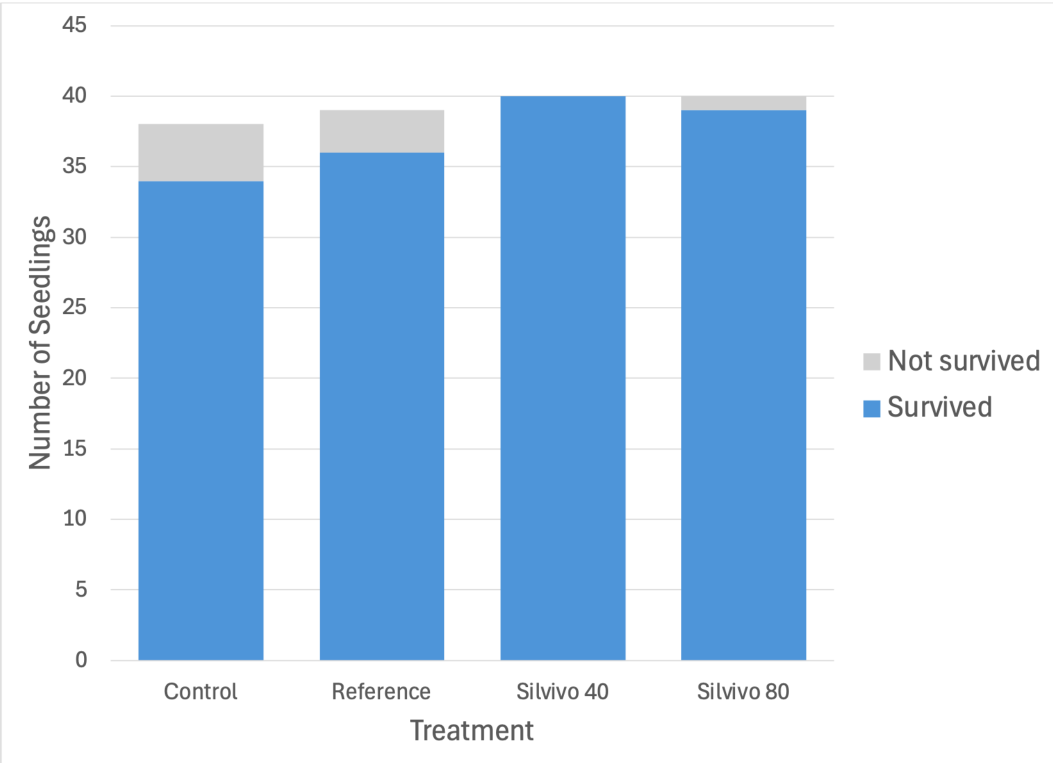

Figure 3. Bar graph of total seedling survival when planted into soil with MSP, n = 38 (Control), n = 39 (Reference) and n = 40 (Silvivo 40 and Silvivo 80). Blue represents the frequency of "survived" and grey represents "not survived". The Fisher's Exact Test (p = 0.090) showed no treatment is significant.

3.1.2 Survival with no MSP

To evaluate the impact of MSP (hypothesis 4), we also quantified survival of the seedlings planted directly into the intact organic layer (Figure 4). Since multiple cell counts were less than 5, a Fisher's Exact Test replaced the standard Pearson's

Chi-Square Test. Figure 4 shows the survival rate of seedlings for all treatments without MSP. The result ($p = 0.001$) indicates there is a significant difference in the survival rate of seedlings grown with no MSP. To determine which treatments are unique, post-hoc pairwise analysis was conducted.

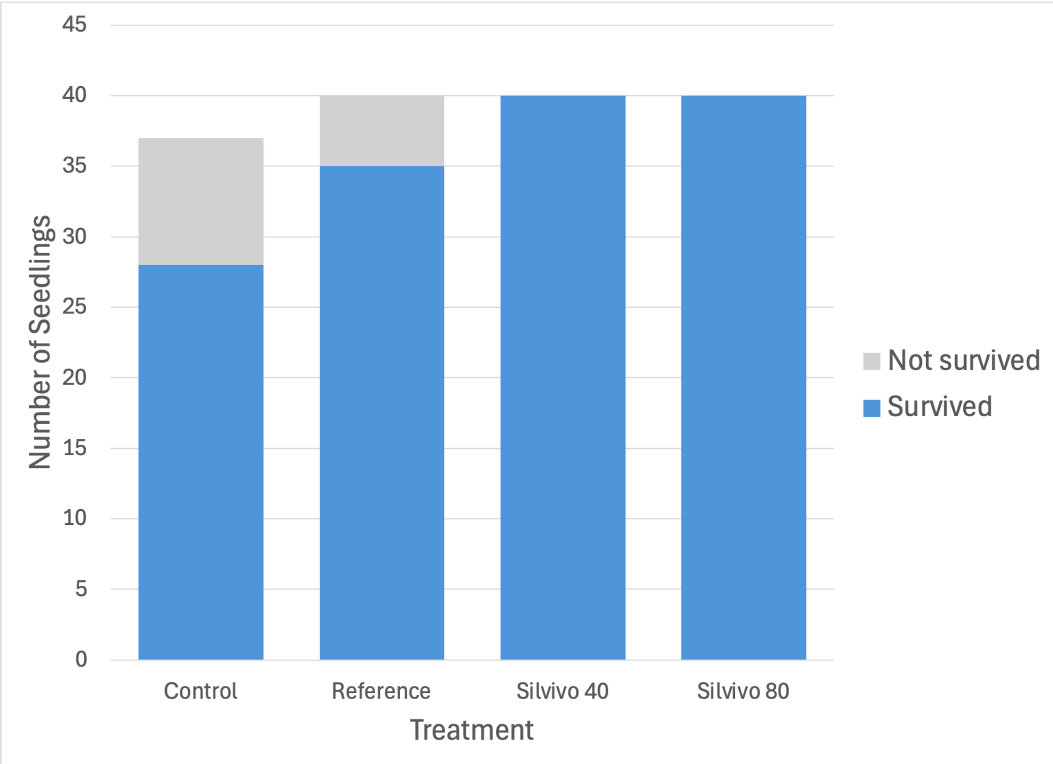

Figure 4. Bar graph of seedling survival when planted into soil without MSP, n = 37 (Control) and n = 40 (Reference, Silvivo 40 and Silvivo 80). Blue represents the frequency of "survived" and grey represents "not survived".

Post-hoc pairwise analysis (Figure 5) reveals which treatments were significantly different. Seedlings treated with Silvivo 40 and Silvivo 80 survived at a significantly higher rate of compared to the Control. Seedlings treated with Silvivo 40 and Silvivo 80 survived at increased rate compared to the Reference. See Table 2.

Table 2. Fisher's Exact Test results for survival with no MSP. Horizontal dashes indicate identical rate of survival. Significant pairs (p< 0.05) are bold.

	Control	Silvivo 40	Silvivo 80	Reference
Reference	p = 0.2403, N = 77	p = 0.0547, N= 80	p = 0.0547, N = 80	X
Silvivo 80	**p = 0.0008, N = 77**	-	X	p = 0.547, N= 80
Silvivo 40	**p = 0.0008, N = 77**	X	-	p = 0.547, N= 80
Control	X	**p = 0.0008, N = 77**	**p = 0.0008, N = 77**	p = 0.2403, N = 77

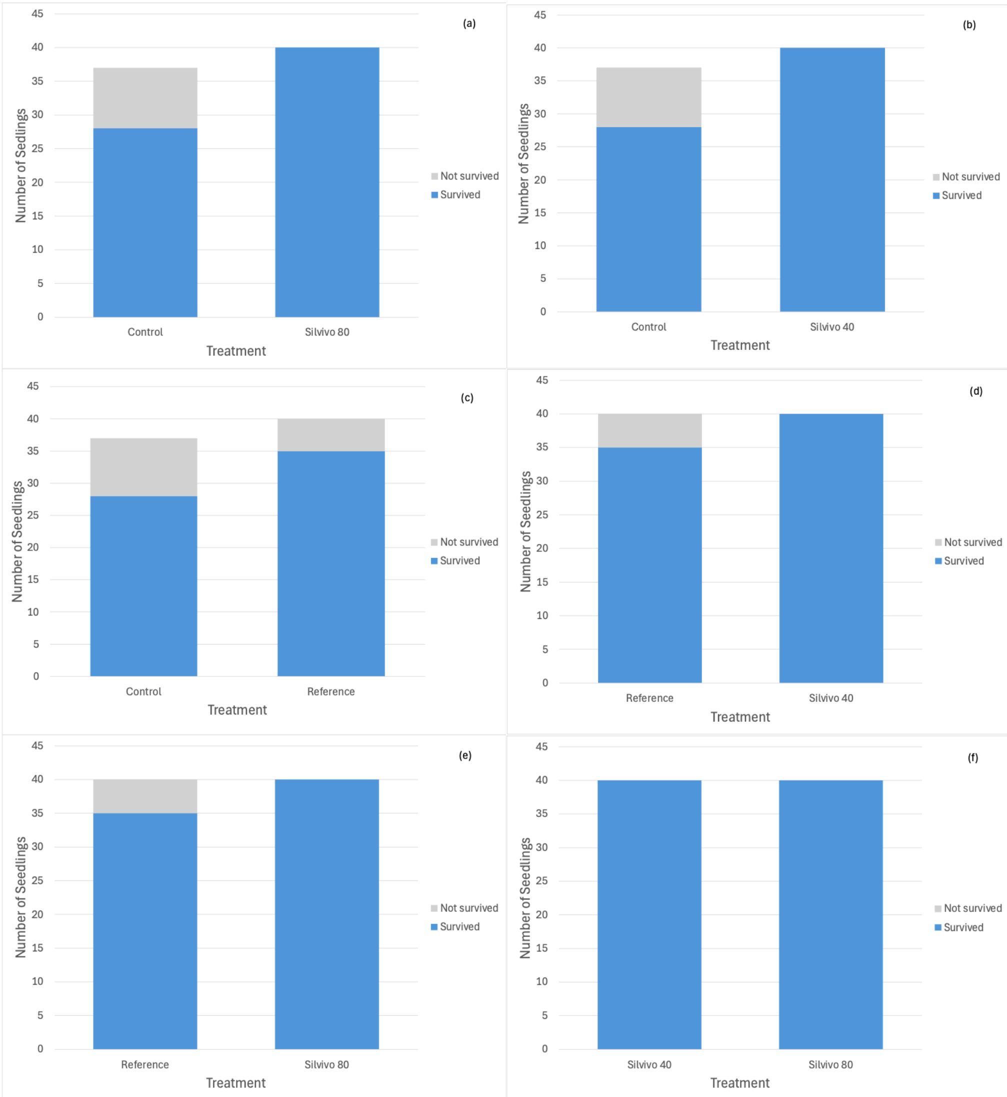

Figure 5. Pairwise contingency comparisons of seedling survival rates across four fertilization treatments after one growing season without MSP, n = 37 (Control) and n = 11140 (Reference, Silvivo 40 and Silvivo 80). Blue represents the frequency of "survive" while grey represents "not survived". Post-hoc pairwise comparisons indicate that treatments of both Silvivo 40 and Silvivo 80 (a,b,d,e) significantly increased the rate of survival compared to the Control and Reference treatments.

3.2 Biomass

Plots 1 and 2 showed signs of browsing and herbivory across seedling in all four treatments. These plots were still evaluated for survival (section 3.1) but were omitted from the analysis of biomass (section 3.2), root collar diameter (section 3.3) and ECM colonization (section 3.4) to avoid skewed results.

The number of seedlings harvested varied in each plot, with at least 3 seedlings per treatment per plot ensuring a minimum of 15 seedlings were harvested per nutritional treatment. The number of seedlings grown with MSP: n = 16 (Control), n = 24 (Reference), n = 22 (Silvivo 40) and n = 20 (Silvivo 80). The number of seedlings grown without MSP n = 15 (Control), n = 15 (Reference) n = 15 (Silvivo 40) and n = 15 (Silvivo 80).

3.2.1 Total Biomass with MSP

Prior to interpreting the data to answer hypothesis 2, it was necessary to evaluate if the data was normally distributed. In Appendix Figure A1, the Shapiro-Wilk test ($p = 0.0012$) indicates that the data is not normally distributed and requires non-parametric evaluation.

The pairwise analysis, using the Wilcoxon method, showed a significant difference in growth of total biomass in response to some treatments. Specifically, Silvivo 40 and Silvivo 80 grew significantly more than the Control ($p = 0.0002$ and $p < 0.0001$, respectively). The Silvivo 40 and Silvivo 80 treatments grew significantly more than the Reference treatment, as well ($p < 0.0001$ and $p < 0.0001$, respectively). See Figure 6.

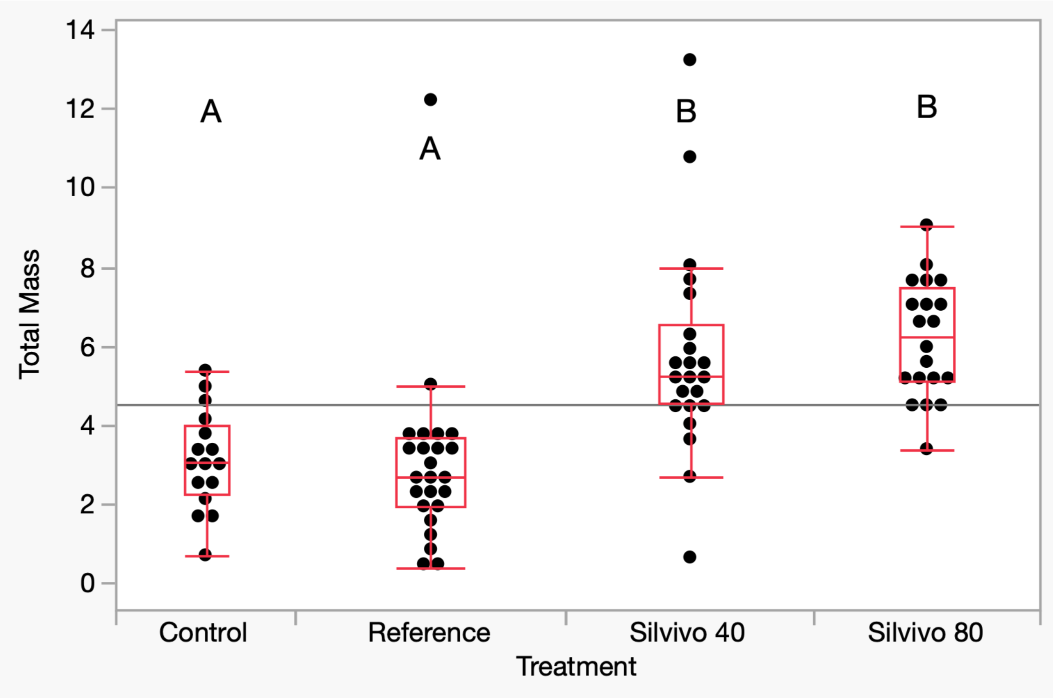

Figure 6. Nonparametric Kruskall-wallis (p <0.05) test evaluating the total biomass of seedling grown in soil with MSP, n = 16 (Control), n = 24 (Reference), n =22 (Silvivo 40) and n = 20 (Silvivo 80). Unique letters indicate significant differences. The horizontal line represents the grand mean.

Figure 7. Pinus sylvestris seedlings harvested after one growing season. The top image displays the treatments of Control (left) and Silvivo 40 (right). The bottom image displays the treatments of Control (left) and Silvivo 80 (right).

3.2.2 Total Biomass with no MSP

Prior to interpreting the data to answer hypothesis 2, it was necessary to evaluate the data for normality. See Appendix Figure A2 for the Shapiro-Wilk test (p = 0.0001) which indicates that the data is not normally distributed and requires non-parametric evaluation.

The pairwise analysis, using the Wilcoxon method, showed a significant difference in growth of total biomass in response to some of the treatments. Specifically, Silvivo 40 and Silvivo 80 grew significantly higher than the Control (p = 0.0004 and p = 0.0008, respectively). The Silvivo 40 and Silvivo 80 grew significantly higher than the Reference treatment, as well (p = 0.0002 and p = 0.0016, respectively). See Figure 8.

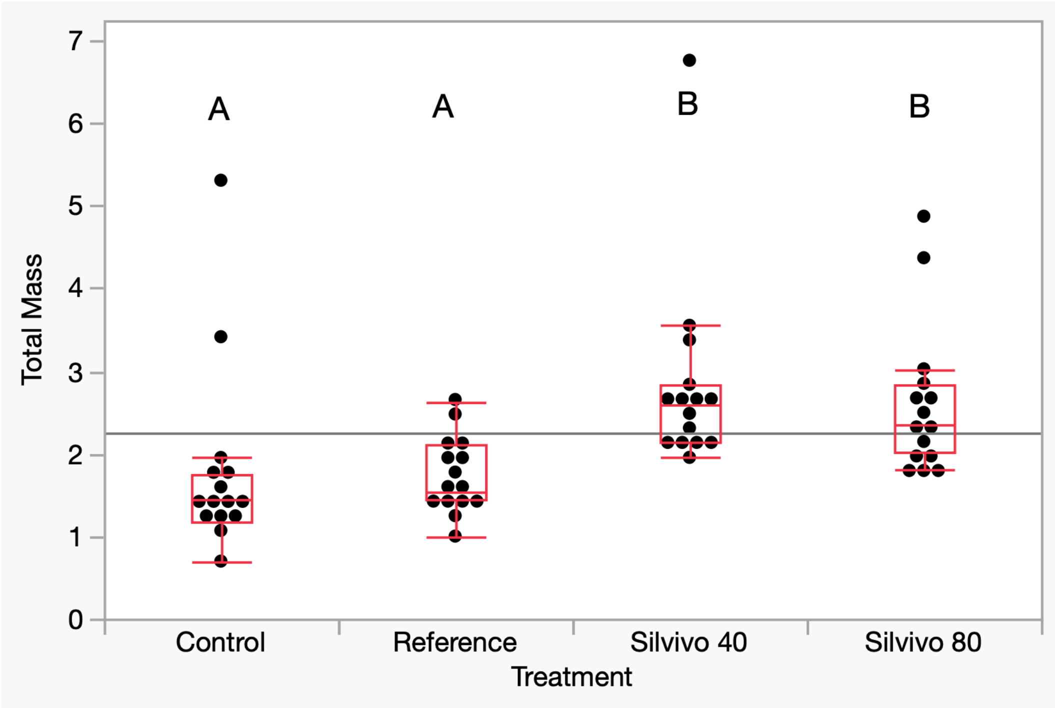

Figure 8. Nonparametric Kruskall-wallis (p <0.05) test evaluating the total biomass of seedlings grown on soil without MSP (n = 15). Unique letters indicate significant differences. The horizontal line represents the grand mean.

3.2.3 R:S ratio with MSP

The Shapiro-Wilk test ($p = 0.0012$; see Appendix Figure A3) confirmed the data is not normally distributed and the Kruskal–Wallis test (df = 3, $p = 0.0003$) confirmed pairwise analysis is necessary to distinguish which treatments are significantly different.

Seedlings treated with Silvivo 80 showed a R:S ratio of 0.24 which is significantly more than the Control (0.19) and Reference (0.14), (p = 0.0248 and 0.0003, respectively). The R:S ratio of seedling treated with Silvivo 40 (0.27) grew significantly more than the Reference ($p = 0.0003$). See Figure 9 for visualization of the variance of R:S ratio in seedlings grown in soil with MSP. See Appendix Table A2 for the complete list of nonparametric pairwise comparisons.

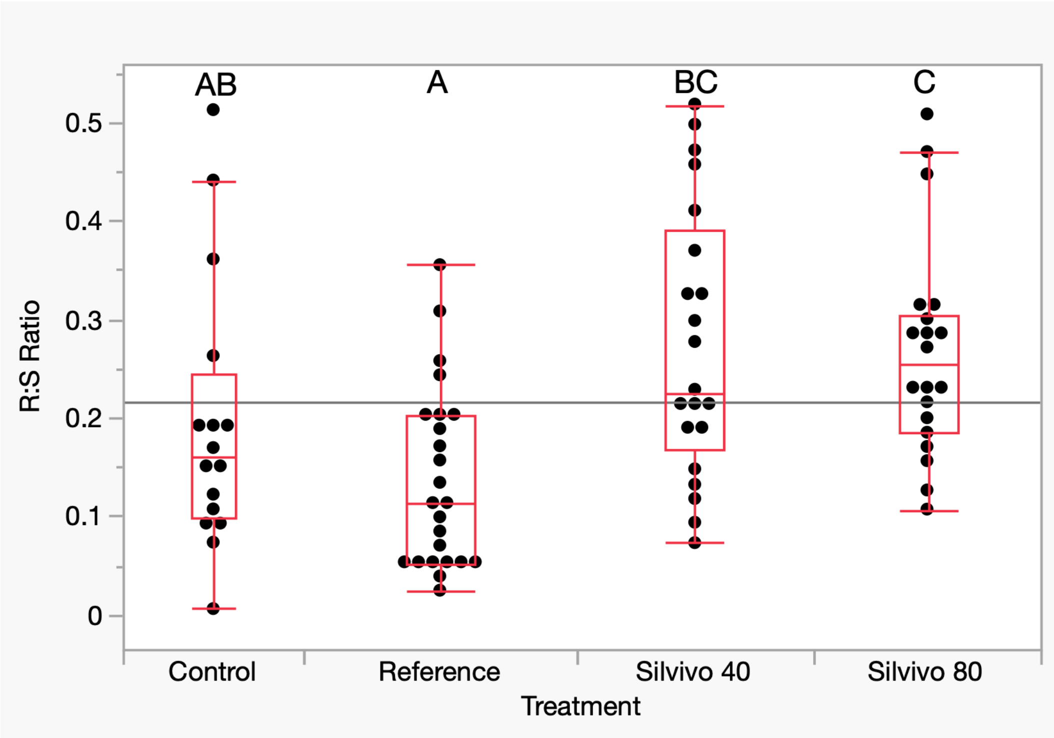

Figure 9. Boxplots showing measurements of R:S Ratio from seedlings grown in soil with MSP n = 16 (Control), n = 24 (Reference), n =22 (Silvivo 40) and n = 20 (Silvivo 80). Unique letters indicate significant differences. The horizontal line represents the grand mean. A Kruskal–Wallis test detected significant differences (df = 3, p = 0.0003).

3.2.4 R:S ratio with no MSP

The Shapiro-Wilk test ($p = 0.0002$; see Appendix Figure A4) revealed the data is not normally distributed and the Kruskal-Wallis test (df = 3, $p = 0.1452$) showed there is no indication that the R:S ratio was different between treatments (Figure 10).

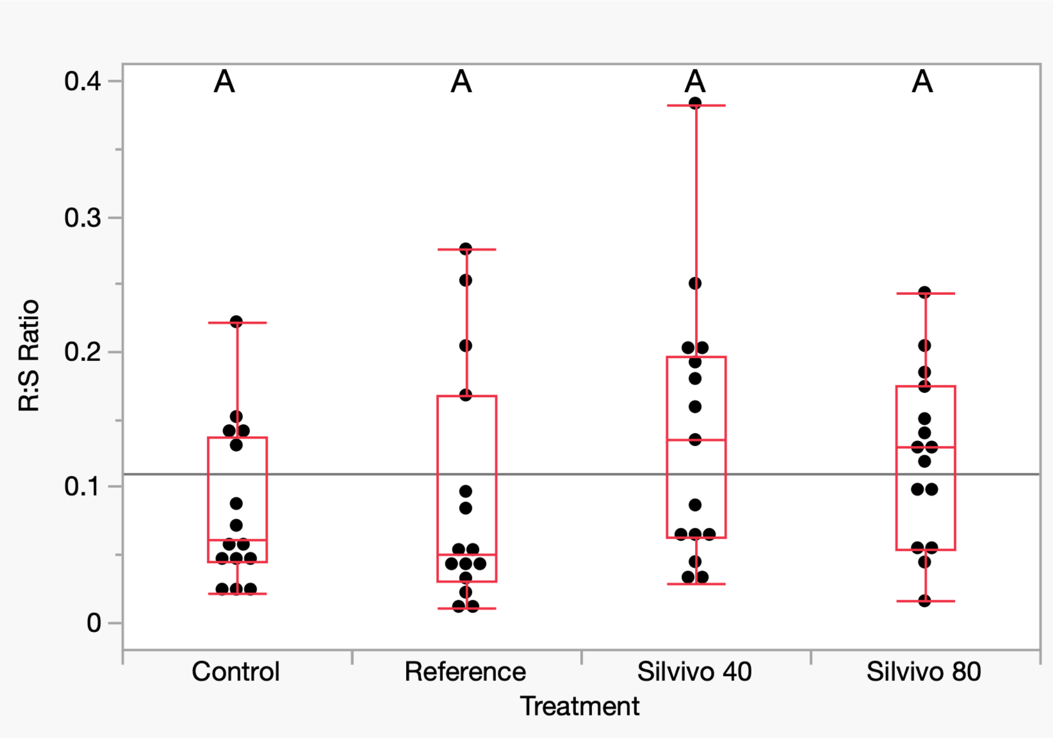

Figure 10. Boxplots showing measurements of R:S ratio from seedlings ground in soil without MSP (n=15). Non-unique letters indicate no significance between treatments. The horizontal line represents the grand mean.

3.3 Root Collar Diameter

3.3.1 RCD with MSP

The Shapiro-Wilk test (p = 0.58) revealed the data is normally distributed and is suitable for parametric analysis (Appendix Figure A5).

A one-way ANOVA showed Silvivo had a significant effect on the RCD of seedlings grown in soil with MSP (dF = 3 , F = 9.5538 , p = 0.0003). Post-hoc Tukey-Kramer HSD tests (Figure 11) indicated that the RCD of seedligns treated with Silivo 40 and Silvivo 80 treatments both grew significantly more than the Control (p = 0.0031 and p = 0.0238, respectively). A similar trend is observed when comparing Silvivo 40 and Silvivo 80 to the Reference treatment (p = 0.0013 and p = 0.0105, respectively). A complete list of the pairwise comparisons is provided in Appendix Table A3.

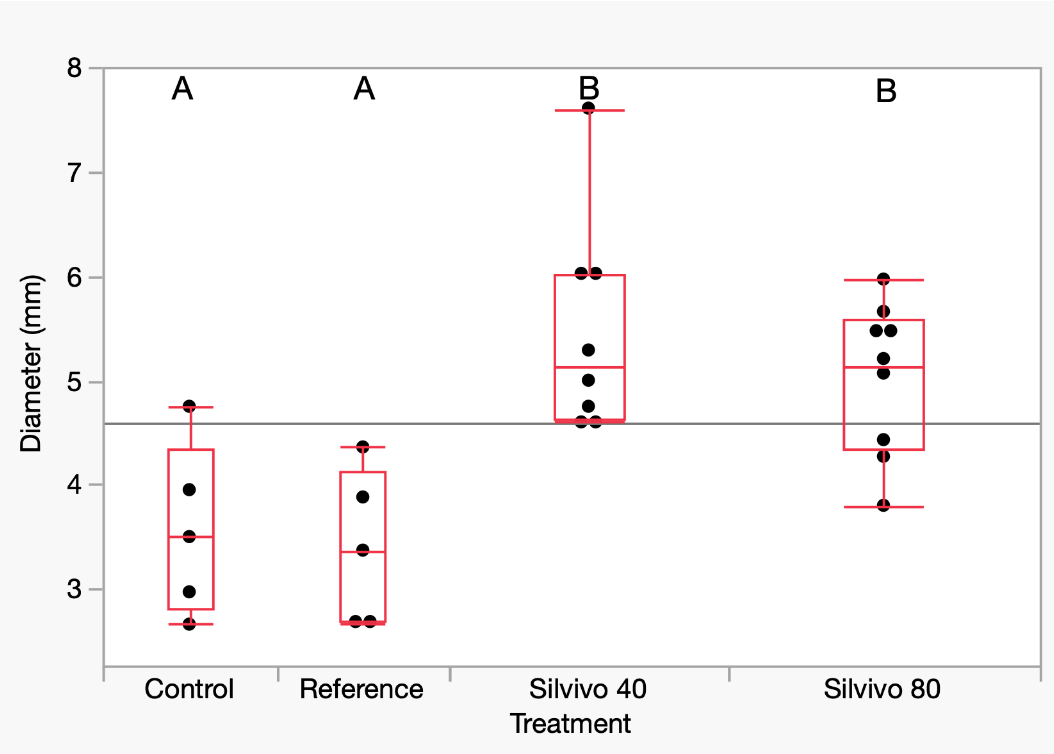

Figure 11. The RCD of Scots pine seedlings grown on soil with MSP, n=5 (Control), m = 5 (Reference), n = 8 (Silvivo 40) and n = 9 (Silvivo 80). Unique letters indicate significant differences (Tukey HSD, p < 0.05). The horizontal line represents the grand mean. The root collars grew significantly more when treated with Silvivo 40 and Silvivo 80 compared to the Control and Reference.

3.3.2 RCD with no MSP

The Shapiro-Wilk test (p = 0.48) revealed the data is normally distributed and is suitable for parametric analysis (see Appendix Figure A6).

A one-way ANOVA showed both Silvivo treatments had a significant effect on the RCD of seedlings grown in soil without MSP (dF = 3, F = 24.6497, p <0.0001). Post-hoc Tukey-Kramer HSD tests indicated that the RCD of seedlings treated with Silvivo 40 and Silvivo 80 treatments grew significantly more than the Control (p <0.0001 and p <0.0001, respectively). A similar trend is observed when comparing Silvivo 40 and Silvivo 80 to the Reference treatment (p <0.0001 and p <0.0001, respectively). See Table Appendix A4 for a complete list of the pairwise comparisons.

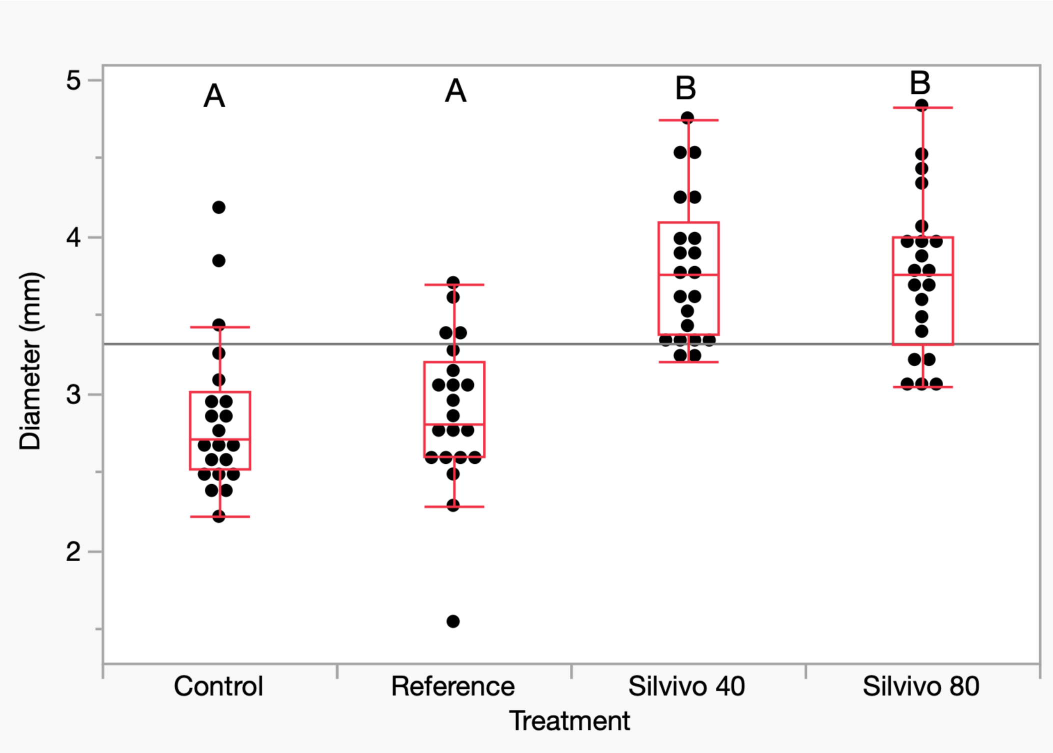

Figure 12. RCD of Scots pine seedlings on soil without MSP (n = 21). Unique letters indicate significant differences (Tukey HSD, p < 0.05). The horizontal line represents the grand mean. The root collars grew significantly more when treated with Silvivo 40 and Silvivo 80 compared to the Control and the Reference treatments.

3.4 Metagenomic Analysis

3.4.1 Sufficient sequencing depth

A rarefaction curve for observed features (Appendix Figure A7) and Shannon diversity (Appendix Figure A8) illustrates how sequencing depth influences the detection of microbial richness and community complexity. In this case, both curves rise steeply at low read counts then plateau, indicating that the sequencing was sufficient to capture majority of the taxa present in the samples. The levelling-off of the observed features curve suggests that additional sequencing would yield few new features, while the stabilization of the Shannon curve shows that most of the underlying diversity is detected.

3.4.2 Richness and diversity

As seen in Figure 13, there was a nonsignificant different in the number of features between CR1 and SR1 ($p = 0.097$). However, there was no significant difference between CR2 and SR2 ($p = 0.851$). There were also no significant differences in the soil samples: CS1-SS1 ($p = 0.476$) and CS2-SS2 ($p = 0.350$). See Appendix Table A5 for all pairwise comparisons.

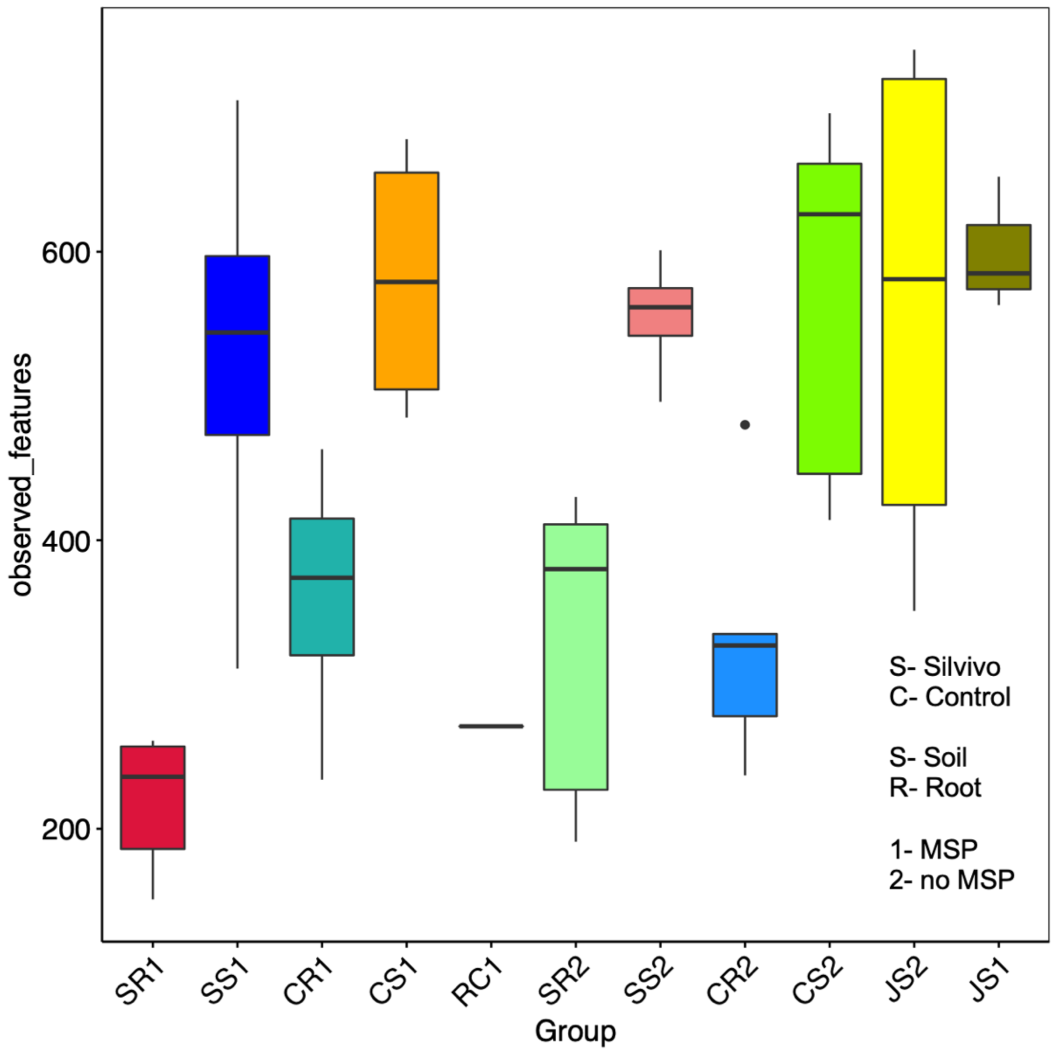

Figure 13. Boxplot of the number of features within the sample groups, (n = 5). The number of unique features of each treatment is captured with each box representing the interquartile range and the median indicated by the central line. Dots signify outliers. Treatment nomenclature example: CS1 represents Control-Soil-MSP. Generated by Novogene.

The Shannon diversity (Figure 14) captures the richness and evenness of feature distribution within each treatment. Lower Shannon diversity indicates lower feature evenness and/or richness. The Silvivo treatment non-significantly alters the Shannon diversity in roots when planted in soil with MSP (CR1-SR1 $p = 0.054$) or without MSP (CR2-SR2 Kruskal-Wallis $p = 0.199$). The same trend is observed in the soil samples, in that Silvivo does not significantly alter the Shannon diversity in soil with MSP (CS1-SS1 (Kruskal-Wallis $p = 0.891$) and without MSP (CS2-SS2 Kruskal-Wallis $p = 0.550$).

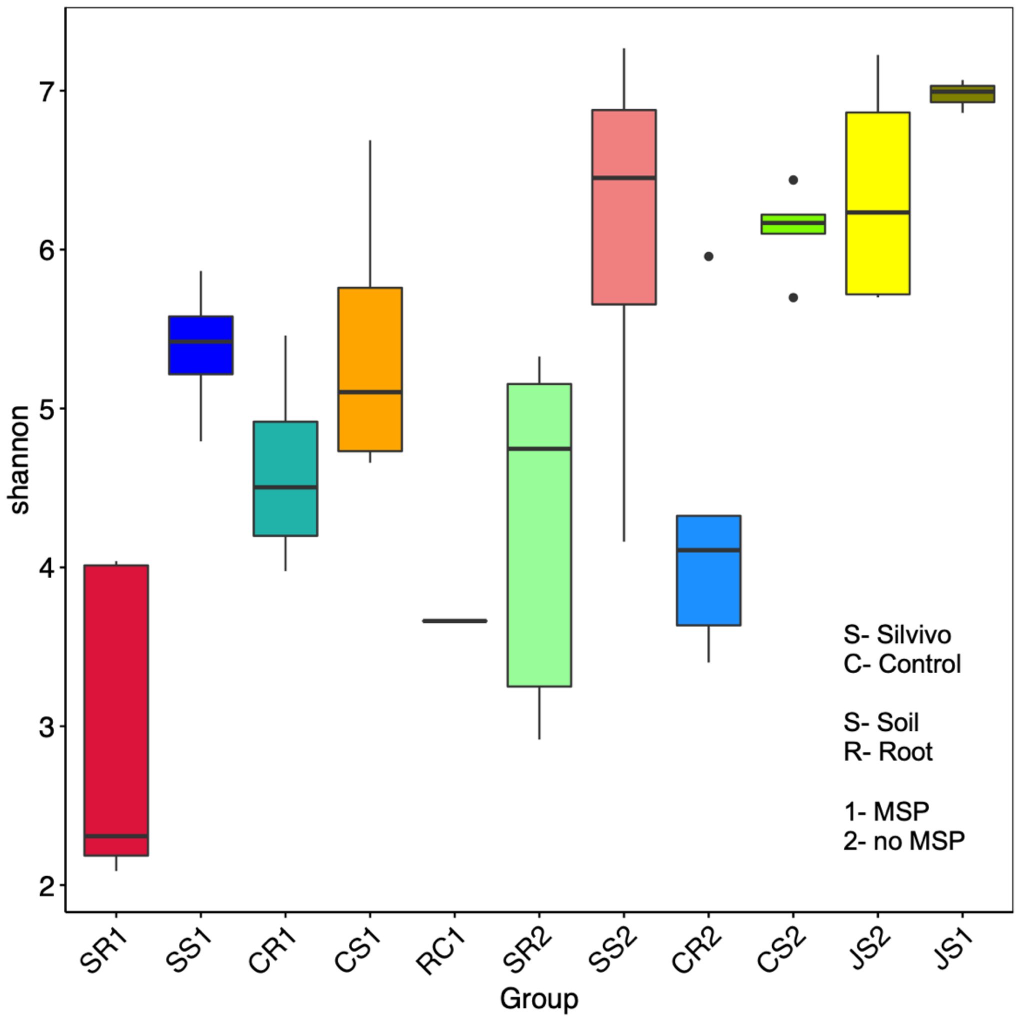

Figure 14. Boxplot of alpha diversity (Shannon diversity) across the sample groups, (n = 5). The distribution of Shannon diversity shows clear distribution of richness and evenness, with each box representing the interquartile range and the median indicated by the central line. Dots signify outliers. Treatment nomenclature example: CS1 represents Control-Soil-MSP. All pairwise comparisons available in Appendix Table A6. Generated by Novogene.

3.4.3 Beta Diversity

Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarities reveal clear compositional differences in fungal communities between treatments, with samples separating along the first principal coordinate axis (PCo1 = 17.89%, PCo2 = 7.83%; Figure 15). PERMANOVA (Appendix Table A7) confirmed which specific treatment pairs showed significantly different composition. SR1 and CR1 formed the tightest clusters but they were not entirely distinct from each other ($p = 0.079$). Additional comparison which compared Silvivo to the control were SR2-CR2, SS1-CS1 and SS2-CS2 ($p = 0.479$, $p = 0.285$ and $p = 0.189$, respectively).

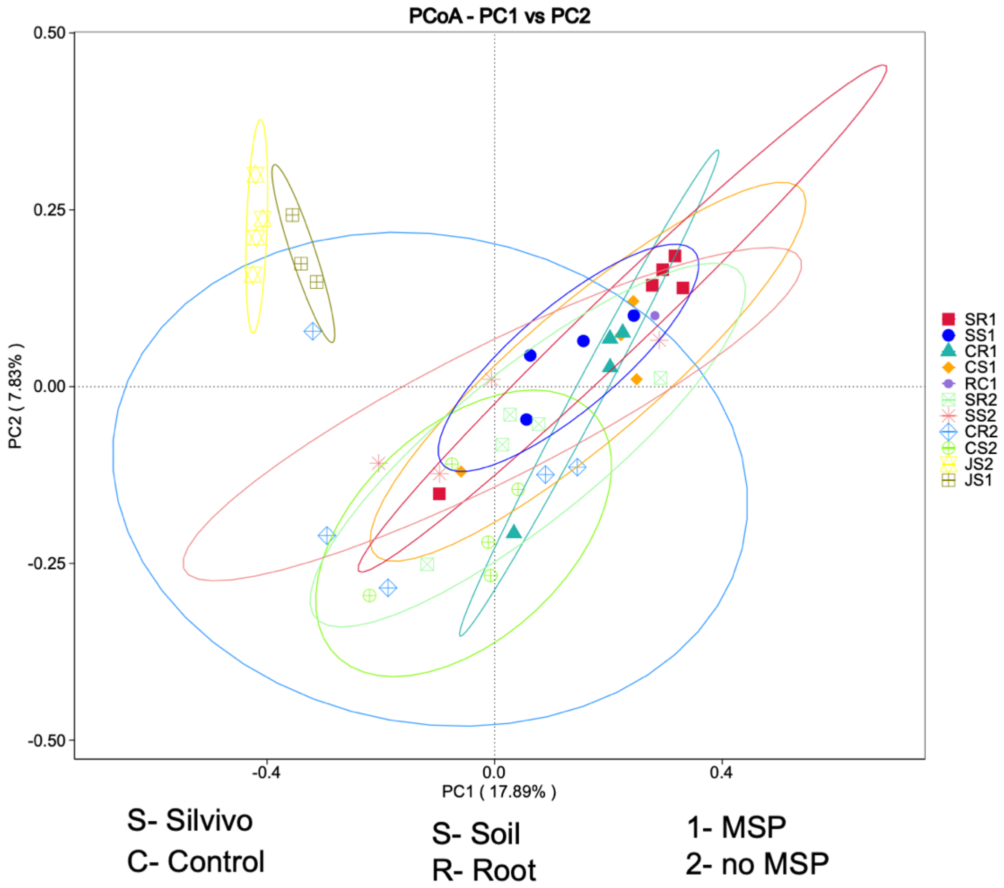

Figure 15. PCoA of Bray-Curtis dissimilarity illustrating the variation in fungal communities across the treatments (n = 5). Each point represents a single sample, with symbols and colours denoting treatment groups. Clustering of points reflects community similarity, with greater dispersion indicating higher within-group variability. Statistically significant separation between treatment groups was confirmed by PERMANOVA (see Appendix Table A7). Treatment nomenclature example: CS1 represents Control-Soil-MSP. Generated by Novogene.

3.4.4 Ten most common functional groups

Key functional guilds represented by the metabarcoding dataset included ECM, saprotrophic and pathogenic fungi. There were also endophytic and ericoid fungi. Figure 16 shows there is a clear trend that ECM often composes the largest proportion of functional guild represented in the data. The relative abundance of the ECM functional guild was lower in JS1 and JS2 compared to the other treatments.

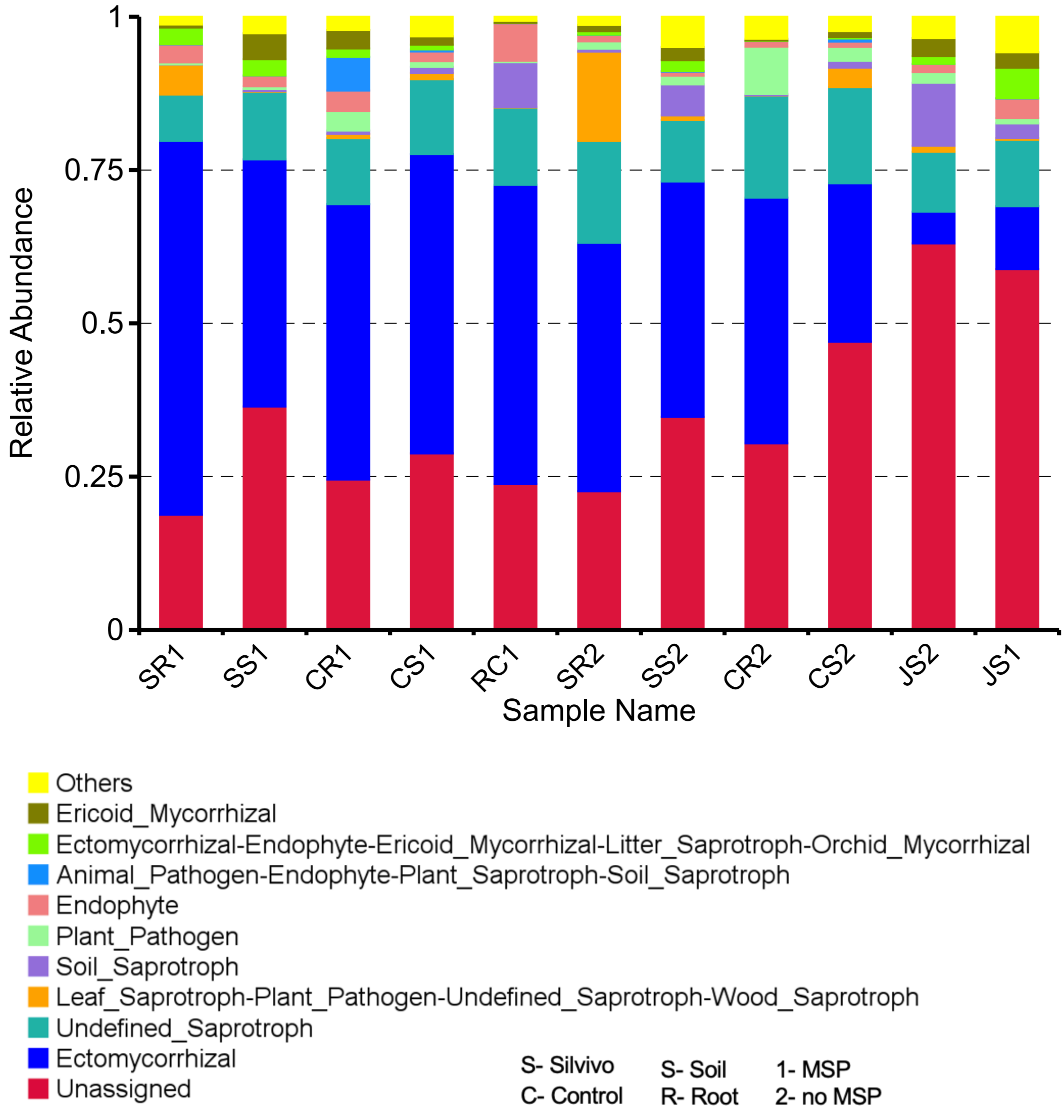

Figure 16. The relative abundance of the 10 most common functional guilds represented by different colored bars (n = 5). Treatment nomenclature example: CS1 represents Control-Soil-MSP. Generated by Novogene.

Pairwise comparisons of fungal community composition across all four treatment combinations were conducted, with the p-values corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate procedure. No statistically significant differences in fungal community structure were detected between any of the treatment pairs (SR1-CR1, SR2-CR2, SS1-CS1 and SS2-CS2) following FDR correction ($q > 0.05$ in all comparisons). In addition, the Plant Pathogen functional group is not enhanced by the Silvivo treatment ($q > 0.05$ in all comparisons).

In Figure 17, the most relatively abundant genus in this comparison is *Thelephora*, except in the treatments JS1 and JS2. However, there was no significant difference in relative abundance of *Thelephora* in any samples take in October (SR1, SS1, CR1, CS1, SR2, SS2, CR2 and CS2) when the p-values were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate procedure. See Appendix Table A8.

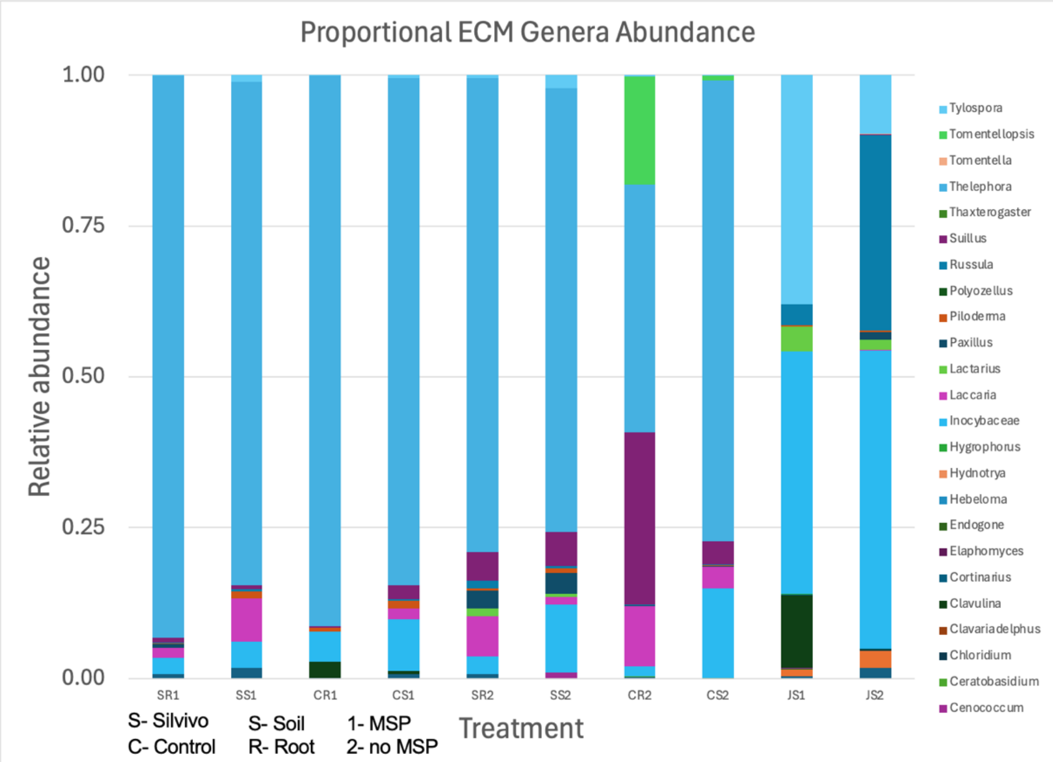

Figure 17. Bar graph of the relative abundance of ECM genera (n = 5). Treatment nomenclature example: CS1 represents Control-Soil-MSP. Adapted from Novogene.

The relative abundances of *Thelephora, Lacarria, Suillus, Lactarius, Russula* and *Piloderma* genera were not significantly different (q >0.05) between the October samples (SR1, SS1, CR1, CS1, SR2, SS2, CR2 and CS2) (see Appendix Tables A8-A13). The population of *Cortinarius* was significantly different between treatments CR1-CR2 (*adj. p* = 0.023).

4. Discussion

The objective of this study was to evaluate the impact of Silvivo, a control-release fertilizer, on Scots pine seedlings by measuring survival and growth metrics. Since early development is critical to seedling survival in the field (Grossnickle 2005; Häggström et al. 2021) and ECM fungi comprise a large percentage of resource acquisition (Smith & Read 2008), there was special interest in understanding if the treatment impacted ECM populations. Finally, we investigated the role of MSP since this is standard silvicultural practice in industrial forestry in Sweden (Öhlund et al. 2025).

4.1 Silvivo impacting survival of Scots pine seedlings

Hypothesis 1 stated Silvivo would increase the survival rate of Scots pine seedling. We tested the N supplementation in two environments: with MSP and without MSP. With MSP, the hypothesis was not supported as the survival was not significantly different between treatments.

However, we observed a significant increase in survival when comparing the treatments on the seedlings grown in soil without MSP. All seedlings treated with either Silvivo 40 or Silvivo 80 survived in this scenario. This was a significantly higher rate of survival compared to the Control treatment, which saw a survival rate of 75.7%. According to the Fisher's Exact Test (Table 2) the Reference treatment did not show a significantly different rate of survival compared the Control, Silvivo 40 or Silvivo 80 ($p = 0.2403$, $p = 0.0547$, and $p = 0.0547$, respectively). The *p*-value of the Treatment compared to both Silvivo 40 and 80 is trending towards significance which suggests there may be an effect on survival rate, which may be more apparent if the sample size is increased. These findings support hypothesis 1, which states Silvivo would improve survival of Scots pine seedlings.

Hypothesis 4, which states Silvivo will improve the measured metrics regardless of MSP, is not supported as Silvivo did not increase survival when grown in soil with MSP. MSP can increase the survival percentage of newly plants seedlings (Collet et al. 2024) which may have a larger impact on survival than the fertilizer treatment, perhaps attributing to similar survival observed between seedlings which did and did not receive nutrient supplementation.

There have been multiple studies focusing on seedling survival, and there are also many factors which contribute to the vitality of a newly planted seedling including but not limited to site index, length of growing season, competing vegetation and root development (Grossnickle 2005; Castro et al. 2021; Häggström et al. 2021; Anthony 2025). Our results show that Silvivo does not improve survival in soil with

MSP but does improve survival in soil without MSP. Häggström et al. (2021) found the opposite but they elaborate that site index impacts treatment efficacy. The site index for this trial was T21 with moderately fertile black soil. A nutrient addition may not be necessary when MSP buries organic material under mineral soil, but when MSP is not utilized Silvivo may have a more pronounced impact on nutrient availability.

4.2 Higher growth among samples treated with Silvivo

4.2.1 Total Biomass

Total seedling biomass served as a key indicator for determining how effectively Silvivo performed across treatments. Hypothesis 2 states seedlings receiving Silvivo will exhibit greater growth than unfertilized seedlings and cause an increase in the ratio of belowground biomass to aboveground biomass. In support of the first part of this hypothesis, seedlings treated with Silvivo 40 and Silvivo 80 both grew significantly more than the Control treatment. Organic N fertilizers may be especially interesting for young seedlings as the uptake of amino acid N may not depend on an exchange of C (Gruffman et al. 2013) allowing the C to be utilized elsewhere in the plant. Organic N fertilizer may also supply a constant supply of N while avoiding leaching due to stronger retention with the surrounding soil (Lim et al. 2022). The Reference treatment, however, did not differ significantly from the Control, which was unexpected given that amino acid fertilizers have been shown to promote growth in seedlings (Majerowicz & Kerbauy 2002; Lim et al. 2022). This may reflect dose timing, nutrient form, or soil interactions specific to the experimental conditions.

4.2.2 Root collar diameter

We measured the RCD of the harvested plants, and in support of hypothesis 2, there is a measurable increase in RCD in the seedlings that were treated with both Silvivo 40 and Silvivo 80 compared to both the Control and the Reference treatments. Interestingly, this same trend was observed in the seedlings grown with and without MSP, which supports hypothesis 4.

Our results corroborated previous studies which suggest there is a measurable increase in diameter after a single growing season in response to fertilization (Rose & Ketchum 2003; Novikova et al. 2023). However, these observations fade and the effect decreases as time progresses. It would require following the trees for multiple growing seasons to understand if Silvivo has a similar effect.

4.2.3 Root to shoot ratio

When comparing N fertilizers from organic and inorganic sources there is a consensus that inorganic N additions favor aboveground growth while organic N additions favor belowground growth (Cambui et al. 2011; Inselsbacher & Näsholm 2012). In addition, different species, as well as different parts of the tree, vary their response pattern to the same fertilization treatment (George et al. 1997; Gruffman et al. 2012) suggesting a tailored nutrient supplementation regime could be beneficial. George et al. (1997) found *Pinus sylvestris* increases root biomass under higher fertility. Our findings only partially align with their results. In soil with MSP, those treated with Silvivo 80 resulted in a higher R:S ratio than both the reference and Control. However, the growth from the Silvivo 40 treatment was not significantly different from that of the Control. In soil without MSP there was no observable difference in the R:S ratio between treatments.

4.3 Shifts in the ectomycorrhizal community

4.3.1 Alpha diversity

Hypothesis 3 states seedlings treated with Silvivo will show altered colonization of ECM than compared to unfertilized seedlings. Figures 13 shows the number of observed features and Figure 14 displays the Shannon diversity. These figures visualize the reactions of the fungal communities to the treatments. The observed features, the number of unique ASVs, was the first method of comparing Silvivo to the Control. There is clearly no indication of significance when seedlings grew in soil without MSP (SR2-CR2, $p = 0.8507$). The seedlings grown in soil with MSP (SR1–CR1, $p = 0.097$) display a trend toward significance**,** suggesting that the Silvivo treatment may, indeed, alter the microbial community. Given the sample size ($n = 5$) it is possible that a larger sample size may reveal a statistically significant effect. Of course, additional testing with larger sampling size would be required.

The same trend is observed when comparing the Shannon diversity. When comparing CR1-SR1, $p = 0.0542$, is trending towards significance which indicates a larger sample size may confirm the hypothesis which states Silvivo impacts the microbial community. The p-value of CR2-SR2, 0.9673, does not suggest a response. Overall, the alpha diversity is not impacted by the treatment. However, we observe the same trend: the alpha diversity of the microbial community may be affected by Silvivo when the seedlings are grown on soil with MSP. This observation is not consistent when MSP is not utilized.

To suggest there is a consistent response in the microbial community to N fertilization is an oversimplification of complex interactions. For instance, inorganic fertilizers often decrease diversity while increasing microbial biomass but organic N supplementation has been shown to maintain, and even increase, both the total biomass and diversity of soil microbes (Dincă et al. 2022). The results of our study agree with these findings relating to organic N. The difference in response could be due to the flux of SOM, which is why inorganic fertilization may be supplemented with organic amendments (Dincă et al. 2022).

4.3.2 Silvivo does not significantly alter the fungi population

Figure 15 is a 2D visualization of the relatedness in community. CR1 and SR1 formed the tightest clusters but they were not entirely distinct from each other ($p = 0.079$), indicating the Silvivo treatment may causes a non-significant shift in the fungal community. Increased N may reduce ECM richness (Lilleskov et al. 2011) which is not necessarily reflected in our findings. In addition, Figure 15 showed an observable difference in fungal population in relation to MSP, which is expected since MSP creates better physical conditions for root penetration and mycorrhizal development (Querejeta et al. 1998). Together these effects may improve seedling performance, but MSP appears to be the dominant driver of the population shift.

Figure 16 visualizes the proportional representation of the ten most common functional guilds among the treatments. ECM are represented in a greater proportion of the functional guilds in SR1 than CR1. An additional interesting finding is pathogenic fungal guilds do not appear to increase in abundance in the Silvivo treatments, contrary to some evidence (Veresoglou et al. 2013; Lekberg et al. 2021) that suggests that increased pathogen abundance could be a side effect of N fertilization.

There could be a shift in ECM genera which would not appear as a shifting functional group. However, the existing research surrounding ECM genera responding to forestry practices is nuanced and Silvivo supplements N which can have mixed results towards ECM genera. *Cortinarius*, *Suillus*, *Tricholoma*, and *Piloderma* consistently show a moderate to strong negative response to elevated N levels (Lilleskov et al. 2011) but this may not always apply when N is inorganic (LeDuc et al. 2013). In contrast, *Thelephora*, *Laccaria*, and *Lactarius* thrive under higher N availability (Lilleskov et al. 2011; LeDuc et al. 2013) while the response from *Russula* heavily depends on which species is present (LeDuc et al. 2013).

Some genera exhibit consistent succession patterns, associating only with younger or older host trees. It is quite common for *Thelephora* to associate with young trees (LeDuc et al. 2013) and is supported by Figure 16.

It is also worth noting that supplemented N, alone, is not accurately representative of the total environmental N and additional sources are likely contributing to mycorrhizal responses (Lilleskov et al. 2011). It is important to note this study did not control or measure the soil N prior to supplementation. The site index (T21), the black soil and the presence of ground vegetation indicate the area is not strongly N deficient. There are other N sources for ECM fungi to utilize outside of the experimental treatment. Understanding this interaction is important for considering where a potential nutrition supplement may be most effective and therefore future research should consider comparing the impact of existing environmental N to Silvivo.

5. Conclusions

H1 is partially supported. Silvivo does not affect the survival of seedlings when planted into soil with MSP. However, Silvivo does improve seedling survival when planted into soil without MSP.

H2 is mostly supported. Seedlings treated with Silvivo increased total biomass and RCD, both when MSP is and is not utilized. However there appears to be less impact on the R:S ratio. In soil without MSP, Silvivo did not appear to impact the R:S ratio. Silvivo which delivered 80mg N did increase the R:S ratio.

H3 is not supported. Figure 15 suggests the fungal community associated with seedlings treated with Silvivo are not significantly different than the Control. In addition, there is no significant shift in the proportion of functional guilds (Figure 16), specifically in relation to ECM. Finally, there appears to be no measurable response in which genera are represented in the ECM community (Figure 17).

H4 is partially supported. Silvivo only increases seedling survival without MSP. Silvivo 80 only impacts the R:S ratio with MSP. Silvivo does not appear to influence the ECM community, and MSP does not impact these observations.

5.1 Limitations and future research

This study has several limitations that should be acknowledged. Foliar and soil nutrients concentrations have not yet been analyzed, which limits the interpretation of nutrient uptake and availability. Furthermore, the relatively small sample size likely limited the statistical power, potentially obscuring biologically meaningful effects. For example, the comparison between SR1 and CR1, in Figures 13 and 14, approached, but did not reach statistical significance. It is possible that a larger sample would provide clearer detection of treatment effects.

Additionally, the statistical analyses did not include the samples from JS1 and JS2. Specifically, ECM relative abundance observed in JS1 and JS2 (Figure 17) was significantly lower than in the other soil treatment groups. This pattern is consistent with ecological expectations and may be explained by two factors: ECM utilize C from host plants (Smith & Read 2008) and since these samples were taken prior to planting the seedlings it is expected this functional group would have a reduced population. ECM fungi typically exhibit seasonal dynamics, such as root colonization during certain periods of the year. For example *Lactarius* typically colonizes root tips at a higher proportion during summer months, likely due to moisture and enhanced C flow from the host (Courty et al. 2008). The sampling window may coincide with a natural low-abundance phase, further contributing to the reduced ECM signal observed in JS1 and JS2. Continued work may focus on quantifying the observations to determine statistical significance.

5.2 Silvicultural implications

Figures 6, 9 and 11 indicate Silvivo combined with MSP can increase growth metrics. Figure 3 shows there is no significant increase in the survival rate when Silvivo is added to soil prepared with MSP. MSP creates conditions to benefit seedlings which may be the more powerful tool for establishing seedlings, compared to a nutrient supplement.

However, MSP is one of many tools available to improve seedling establishment. Where MSP is not implemented or unsuitable, Silvivo may still provide benefits. In unprepared soils, it may increase the rate of survival (Figure 2), total biomass (Figure 8) and the diameter of the root collar (Figure 12). An important consideration is that MSP affects the seedling growth and survival in dimensions beyond nutrition such as water accessibility (Nilsson et al. 2019), removal of competitive vegetation (Löf et al. 2012) and the increased protection against the pine weevil. These factors are heavily influenced by geography and since this study focused on one tree species in one soil type it would be incomplete and short-sighted to suggest a forest manager prescribe Silvivo in place of MSP.

MSP is simply a tool, as is nutritional supplementation, and it is of great benefit for responsible and sustainable forest management to consider which tool is most appropriate to address specific conditions and challenges.

There is a plethora of ecological interactions playing into seedling establishment. Further exploration into these dynamics could indicate more conditions and scenarios in which nutritional supplementation is an appropriate silvicultural tool. Expanding the scope to other commercially important species in Swedish forestry, i.e. Norway spruce and birch, would clarify whether such findings can be applied to a broader reforestation plan (Gruffman et al. 2012). The trend regarding ECM shifts would benefit from inoculation experiments with controlled fungal communities to establish whether these shifts are a driver or a consequence of altered seedling performance. Finally, given the increasing frequency of drought stress under projected Scandinavian climate scenarios, assessing how fertilized seedlings respond to water limitation would contribute to knowledge of resilience to better inform reforestation planning under a changing climate.

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Popular science summary

Every year, vast stretches of boreal forest in Sweden are harvested and need to be replanted. It's not guaranteed, however, that these young trees will survive the first months planted in the field. Nutrients are limited and the seedlings face a tough start. So, what can we do to improve their chances? One potential solution is fertilization.

We applied Silvivo, an organic nitrogen fertilizer, to Scots pine freshly planted seedlings in northern Sweden. We also tested whether mechanically loosening the soil through a technique called mounding, impacted how well Silvivo worked. After one full growing season, from June to October, we measured how well the seedlings had grown and survived.

The results were encouraging. Seedlings treated with Silvivo grew more compared to untreated seedlings. Perhaps most interestingly, the fertilizer improved survival rates in plots where the soil had *not* been mechanically prepared. This could indicate that Silvivo acts as a safety net on challenging sites, where mechanical soil preparation cannot be used.

Tree roots don't work alone. They form partnerships with fungi called ectomycorrhizal fungi, which help trees absorb water and nutrients. We used DNA sequencing to identify which fungal communities were living on the seedlings' roots and if Silvivo changed which fungi grew.

Interestingly, it didn't. The fungal communities looked similar between fertilized and unfertilized seedlings. This was unexpected, since fertilizers often affect which fungi can thrive. But it also offers a reassuring message: the fertilizer improved tree growth without disrupting these ecologically important underground partnerships.

The trees survive more and grow better with Silvivo, but we can't say this is caused by a shift in the fungal community, at least not entirely. Therefore, the ectomycorrhizal community is probably one of many influences that cause better tree growth. These findings suggest that Silvivo could be a tool for improving pine reforestation. They also highlight a practical insight for forest managers: Matching the right treatment to the right conditions could make reforestation efforts more effective across Sweden and similar northern landscapes.

Acknowledgements

I would like to express my deep gratitude to my primary supervisor, Sajeevan Radha Sivarajan. His insightful support and guidance were essential to completing this project. A special thanks Jonathan Love and Cropcision AB for leading the hours of work in the field. I also thank Stephan Hupperts for his expertise regarding mycorrhizal fungi. In addition, I also want to thank Edoardo Piombo, from Umeå Plant Science, for providing invaluable guidance in interpreting the metabarcoding data.

The guidance, feedback and support were essential to completing a project which I am truly proud to present.

Appendix 1

Table A1. Fisher's Exact Test results for survival with no MSP. Signifiant pairs (p< 0.05) are bold.

	Control	Silvivo 40	Silvivo 80	Reference
Reference	*p* = 0.2403, N = 77	*p* = 0.547, N= 80	*p* = 0.0547, N = 80	X
Silvivo 80	***p* = 0.0008, N = 77**	-	X	*p* = 0.547, N= 80
Silvivo 40	***p* = 0.0008, N = 77**	X	-	*p* = 0.547, N= 80
Control	X	***p* = 0.0008, N = 77**	***p* = 0.0008, N = 77**	*p* = 0.2403, N = 77

Table A2. Nonparametric comparison of R:S Ratio of seedlings grown in soil without MSP using Wilcoxon method. Signifiant pairs (p< 0.05) are bold.

Treatment	**- Treatment**	**p-value**
Silvivo 80	**Reference**	**0.0003**
Silvivo 40	**Reference**	**0.0005**
Silvivo 80	**Control**	**0.0248**
Silvivo 40	Control	0.0594
Silvivo 80	Silvivo 40	0.9896
Reference	Control	0.1897

Table A3. Parametric comparison of RCD of seedlings grown in soil with MSP using Tukey-Kramer HSD. Signifiant pairs (p< 0.05) are bold.

Treatment	**- Treatment**	**p-value**
Silvivo 40	**Reference**	**0.0013**
Silvivo 40	**Control**	**0.0031**
Silvivo 80	**Reference**	**0.0105**
Silvivo 80	**Control**	**0.0238**
Silvivo 40	Silvivo 80	0.6948
Control	Reference	0.9887

Table A4. Parametric comparison of RCD of seedlings grown in soil without MSP using Tukey-Kramer HSD. Signifiant pairs (p < 0.05) are bold.

Treatment	**- Treatment**	**p-value**
Silvivo 40	**Control**	**<0.0001**

Treatment	- Treatment	p-value
Silvivo 40	**Reference**	**<0.0001**
Silvivo 80	**Control**	**<0.0001**
Silvivo 80	**Reference**	**<0.0001**
Silvivo 40	Silvivo 80	0.9967
Reference	Control	0.9970

Table A5. P-values of the pairwise comparisons of Observed features. Signifiant pairs (p< 0.05) are bold. Adapted from Novogene.

Pair	p-value
CR1 - CR2	0.7833
CR1 - CS1	**0.0069**
CR1 - CS2	**0.0078**
CR1 - JS1	**0.0055**
CR1 - JS2	**0.0116**
CR1 - SR1	0.097
CR1 - SR2	0.6513
CR1 - SS1	**0.0379**
CR1 - SS2	**0.0129**
CR2 - CS1	**0.0022**
CR2 - CS2	**0.0023**
CR2 - JS1	**0.002**
CR2 - JS2	**0.004**
CR2 - SR1	0.1386
CR2 - SR2	0.8507
CR2 - SS1	**0.0153**
CR2 - SS2	**0.0045**
CS1 - CS2	0.8383
CS1 - JS1	0.7652
CS1 - JS2	0.8333
CS1 - SR1	**0**
CS1 - SR2	**0.0014**
CS1 - SS1	0.4759
CS1 - SS2	0.8007
CS2 - JS1	0.6177
CS2 - JS2	0.9858
CS2 - SR1	**0**
CS2 - SR2	**0.0014**
CS2 - SS1	0.583
CS2 - SS2	0.9505
JS1 - JS2	0.6222

JS1 - SR1	**0**
JS1 - SR2	**0.0013**
JS1 - SS1	0.3397
JS1 - SS2	0.5951
JS2 - SR1	**0.0001**
JS2 - SR2	**0.0025**
JS2 - SS1	0.6141
JS2 - SS2	0.9664
SR1 - SR2	0.1933
SR1 - SS1	**0.0003**
SR1 - SS2	**0.0001**
SR2 - SS1	**0.0099**
SR2 - SS2	**0.0028**
SS1 - SS2	0.6438

Table A6. P-values of the pairwise comparisons for Shannon divesity. Signifiant pairs (p< 0.05) are bold. Adapted from Novogene.

Group	p-value
CR1 - CR2	0.7499
CR1 - CS1	0.1615
CR1 - CS2	**0.0028**
CR1 - JS1	**0.0002**
CR1 - JS2	**0.0019**
CR1 - SR1	**0.0542**
CR1 - SR2	0.7209
CR1 - SS1	0.1258
CR1 - SS2	**0.0057**
CR2 - CS1	0.0761
CR2 – CS2	**0.0007**
CR2 - JS1	**0**
CR2 - JS2	**0.0005**
CR2 - SR1	0.0848
CR2 - SR2	0.9673
CR2 - SS1	0.0564
CR2 - SS2	**0.0016**
CS1 - CS2	0.0959
CS1 - JS1	0.0064
CS1 - JS2	0.0609
CS1 - SR1	**0.0013**
CS1 - SR2	0.0704
CS1 - SS1	0.8906
CS1 - SS2	0.1369
CS2 - JS1	0.1518
CS2 - JS2	0.7426

CS2 - SR1	**0**
CS2 - SR2	**0.0006**
CS2 - SS1	0.1264
CS2 - SS2	0.9153
JS1 - JS2	0.2741
JS1 - SR1	**0**
JS1 - SR2	**0**
JS1 - SS1	**0.0089**
JS1 - SS2	0.1439
JS2 - SR1	**0**
JS2 - SR2	**0.0004**
JS2 - SS1	0.0807
JS2 - SS2	0.6802
SR1 - SR2	0.092
SR1 - SS1	**0.0009**
SR1 - SS2	0
SR2 - SS1	0.052
SR2 - SS2	**0.0014**
SS1 - SS2	0.175

Table A7. Results of permutational multivariate analysis of variance (PERMANOVA) examining the effect of fertilization treatment on ECM community composition. Signifiant pairs (p < 0.05) are bold. Adapted from Novogene.

Group	F.Model	R2	Pr(>F)
SR1-SS1	1.58173	0.18431(0.81569)	0.114
SR1-CR1	1.55177	0.18146(0.81854)	0.079
SR1-CS1	1.6147	0.18744(0.81256)	0.097
SR1-SR2	1.6735	0.173(0.827)	0.063
SR1-SS2	1.81945	0.2063(0.7937)	0.086
SR1-CR2	**2.20451**	**0.21603(0.78397)**	**0.027**
SR1-CS2	**3.15271**	**0.28269(0.71731)**	**0.007**
SR1-JS2	**5.41472**	**0.43615(0.56385)**	**0.008**
SR1-JS1	**4.01174**	**0.4007(0.5993)**	**0.014**
SS1-CR1	1.10085	0.15503(0.84497)	0.219
SS1-CS1	1.08371	0.15299(0.84701)	0.285
SS1-SR2	1.06796	0.13237(0.86763)	0.326
SS1-SS2	1.00457	0.14342(0.85658)	0.435
SS1-CR2	1.54542	0.18085(0.81915)	0.069
SS1-CS2	**1.77889**	**0.20263(0.79737)**	**0.005**
SS1-JS2	**3.97678**	**0.3986(0.6014)**	**0.024**
SS1-JS1	**2.54046**	**0.33691(0.66309)**	**0.023**
CR1-CS1	0.94949	0.13663(0.86337)	0.597
CR1-SR2	1.02883	0.12814(0.87186)	0.392

CR1-SS2	1.11848	0.15712(0.84288)	0.278
CR1-CR2	1.49158	0.17565(0.82435)	0.065
CR1-CS2	**1.82843**	**0.20711(0.79289)**	**0.007**
CR1-JS2	**3.92234**	**0.3953(0.6047)**	**0.026**
CR1-JS1	**3.10742**	**0.38328(0.61672)**	**0.028**
CS1-SR2	1.35757	0.16244(0.83756)	0.052
CS1-SS2	1.10786	0.15586(0.84414)	0.322
CS1-CR2	1.65473	0.19119(0.80881)	0.051
CS1-CS2	**1.76394**	**0.20127(0.79873)**	**0.026**
CS1-JS2	**4.3456**	**0.42004(0.57996)**	**0.029**
CS1-JS1	**3.10824**	**0.38334(0.61666)**	**0.028**
SR2-SS2	0.89165	0.11299(0.88701)	0.674
SR2-CR2	0.97897	0.10903(0.89097)	0.479
SR2-CS2	**1.33011**	**0.14256(0.85744)**	**0.068**
SR2-JS2	**3.29047**	**0.31976(0.68024)**	**0.006**
SR2-JS1	**2.6833**	**0.30902(0.69098)**	**0.023**
SS2-CR2	1.08065	0.13373(0.86627)	0.239
SS2-CS2	1.09451	0.13522(0.86478)	0.189
SS2-JS2	**2.4905**	**0.29333(0.70667)**	**0.028**
SS2-JS1	**1.98669**	**0.28435(0.71565)**	**0.023**
CR2-CS2	1.00483	0.11159(0.88841)	0.451
CR2-JS2	**2.17202**	**0.23681(0.76319)**	**0.021**
CR2-JS1	**1.71749**	**0.22254(0.77746)**	**0.022**
CS2-JS2	**3.38502**	**0.32595(0.67405)**	**0.012**
CS2-JS1	**2.74862**	**0.31418(0.68582)**	**0.033**
JS2-JS1	**1.91386**	**0.27682(0.72318)**	**0.033**

Table A8. MetagenomeSeq differential abundance analysis of Thelephora (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.

Pair	logFC	se	*p*-value	adj. *p*
SR1-CR1	-0.7538333	0.8704873	0.38649566	1
CR2-CS2	0.17376098	1.08496406	0.87276002	1
SR2-CR2	-1.1768848	1.14424593	0.30370324	1
CS1-CS2	-0.8758154	0.9616535	0.36243288	0.92445641
SR1-SR2	-0.8115484	0.93375482	0.38477951	1
SR2-SS2	-1.0213599	1.03226898	0.32245187	1
SS1-CS1	0.16388009	0.89626077	0.85491676	1
SS1-SS2	-0.6967163	1.00802242	0.48945782	1
SS2-CS2	0.03474701	0.88675752	0.96874342	1
CR1-CR2	-1.2460987	1.06870263	0.24361767	1

Table A9. MetagenomeSeq differential abundance analysis of Laccaria (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.

Pair	logFC	se	*p*-value	adj. *p*
SR1-CR1	-4.6431669	2.77316003	0.09406705	1
CR2-CS2	-1.2158619	2.21154742	0.58247109	1
SR2-CR2	1.24886026	2.51401703	0.6193591	1
CS1-CS2	1.2270312	1.7609172	0.4859194	1
SR1-SR2	-0.3251231	2.75278607	0.90598293	1
SR2-SS2	4.36792308	3.01631358	0.14758914	0.90809636
SS1-CS1	-0.8734929	2.43800998	0.72013297	1
SS1-SS2	1.74735345	3.03102336	0.5642843	1
SS2-CS2	-0.5985031	2.20847327	0.78638866	1
CR1-CR2	2.7284131	2.20845253	0.21666637	1

Table A10. MetagenomeSeq differential abundance analysis of Suillus (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and *without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.*

Pair	**logFC**	**se**	***p*-value**	**adj. *p***
SR1-CR1	1.97034351	1.56075311	0.20679365	1
CR2-CS2	-0.5114484	2.70204354	0.84987176	1
SR2-CR2	-0.1226332	2.99656429	0.96735601	1
CS1-CS2	-1.9749341	1.36494367	0.14792552	0.83472257
SR1-SR2	2.18625745	2.10630671	0.29928971	1
SR2-SS2	1.3176752	2.01864742	0.51391647	1
SS1-CS1	1.46590525	1.00240115	0.14363332	1
SS1-SS2	1.69077872	1.01198807	0.09477114	1
SS2-CS2	-2.043991	1.46415185	0.1627073	1
CR1-CR2	0.15478192	3.17631137	0.9611344	1

Table A11. MetagenomeSeq differential abundance analysis of Lactarius (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.

Pair	**logFC**	**se**	***p*-value**	**adj. *p***
SR1-CR1	0.1065918	1.18418673	0.92827715	1
CR2-CS2	0.1589738	0.43576139	0.71524693	1
SR2-CR2	-1.330232	1.46021682	0.36230458	1
CS1-CS2	-0.5090107	0.66866377	0.44651635	1
SR1-SR2	1.00130345	1.61592305	0.53548957	1
SR2-SS2	0.92453848	3.06888088	0.7632144	1
SS1-CS1	-1.4160158	0.92609188	0.12625874	1
SS1-SS2	1.36517135	1.15247661	0.23619364	1
SS2-CS2	-2.1024407	1.37531448	0.1263393	1
CR1-CR2	-0.3121433	0.4259275	0.46364656	1

Table A12. MetagenomeSeq differential abundance analysis of Cortinarius (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.

Pair	logFC	se	*p*-value	adj. *p*
SR1-CR1	-0.0213004	0.58417947	0.97091385	1
CR2-CS2	0.6583881	0.58518292	0.26054753	1
SR2-CR2	0.23630743	0.23408161	0.31273071	1
CS1-CS2	-1.1497164	1.1418432	0.31398515	0.92445641
SR1-SR2	-0.3260732	0.2836866	0.25038548	1
SR2-SS2	1.01671133	0.33260115	0.00223677	0.17670505
SS1-CS1	1.17012754	0.98788481	0.23622402	1
SS1-SS2	0.09465141	0.99482962	0.92420098	1
SS2-CS2	-0.5307491	0.82170709	0.51833744	1
CR1-CR2	-0.1530529	0.34191351	0.65441579	1

Table A13. MetagenomeSeq differential abundance analysis of Russula (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.

Pair	logFC	se	*p*-value	adj. *p*
SR1-CR1	-1.9006313	1.39709135	0.173697	1
CR2-CS2	1.19222078	1.47305937	0.41831378	1
SR2-CR2	-1.6654182	1.34490592	0.21559899	1
CS1-CS2	-1.4706236	1.10765997	0.18428218	0.92445641
SR1-SR2	-0.0337417	167.42155	0.9998392	1
SR2-SS2	-1.0879886	1.60732368	0.49847287	1
SS1-CS1	-0.2233073	1.09401354	0.83826167	1
SS1-SS2	-0.9919862	1.22208099	0.41695295	1
SS2-CS2	-0.6671387	1.22332253	0.58551298	1
CR1-CR2	-0.3342501	0.88635533	0.70609466	1

Table A13. MetagenomeSeq differential abundance analysis of Piloderma (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Adapted from Novogene.

Pair	logFC	se	*p*-value	adj. *p*
SR1-CR1	-1.4447384	1.20635113	0.23106881	1
CR2-CS2	1.36669783	1.47102296	0.35284769	1
SR2-CR2	-0.5535999	1.44684658	0.70199771	1
CS1-CS2	-1.9106875	1.26474459	0.13085727	0.79520957
SR1-SR2	-2.2408879	1.44474878	0.12088799	1
SR2-SS2	0.40057547	1.87624626	0.83093828	1
SS1-CS1	0.20159348	1.46917721	0.89086068	1
SS1-SS2	0.35156161	1.2388776	0.77658336	1
SS2-CS2	-2.0303395	0.86484983	0.01889375	0.90370696
CR1-CR2	-2.4141439	1.26503127	0.05634381	1

Table A14. MetagenomeSeq differential abundance analysis of Tylospora (genus level) across treatment pair comparisons. Log fold change (logFC), standard error (se), p-value, and FDR-adjusted p-value (adj. P) are reported for pairwise comparisons between Silvivo and Control treatments across root and soil sample types with MSP(1) and without MSP (2). No treatment pairs met the significance threshold of logFC > 1 and adj.p-value < 0.05, indicating no statistically significant difference in the relative abundance of Thelephora between any compared groups. SR = Silvivo root; SS = Silvivo soil; CR = Control root; CS = Control soil. 1 = MSP. 2 = no MSP. Bold indicates significant value. Adapted from Novogene.

Pair	logFC	se	*p*-value	adj. *p*
SR1-CR1	-3.4457919	1.29335253	0.00771642	1
CR2-CS2	-1.3246892	1.10835491	0.23201476	1
SR2-CR2	-0.6028247	1.099913	0.58364674	1
CS1-CS2	-2.2649164	1.17299734	0.05349797	0.72586422
SR1-SR2	0.35553705	1.12186282	0.75130682	1
SR2-SS2	0.27962748	1.42611051	0.84454989	1
SS1-CS1	0.43051158	1.34797288	0.74944062	1
SS1-SS2	0.44490746	1.48355601	0.76425908	1
SS2-CS2	-2.1538254	1.43615975	0.13368913	1
CR1-CR2	**2.50060639**	**0.6175929**	**5.14E-05**	**0.02845**

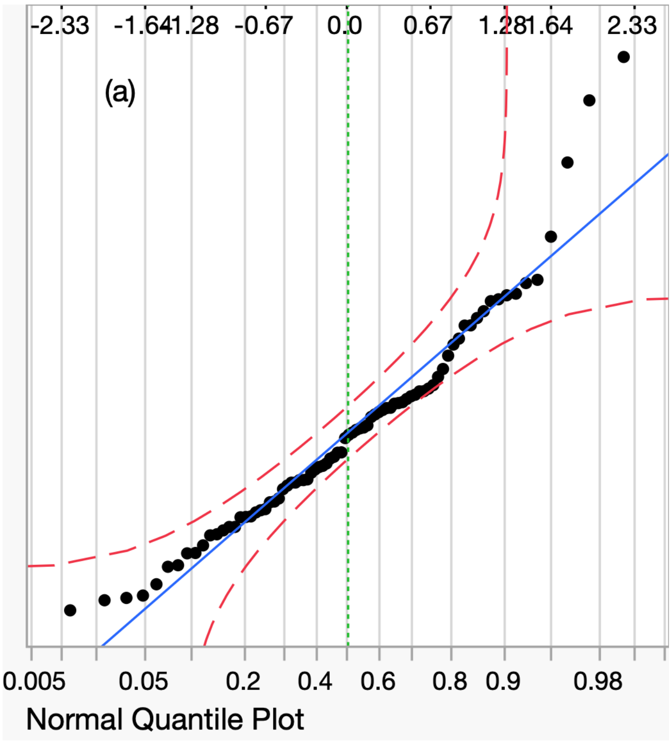

Figure A1. Q-Q plot of total biomass data or seedlings grown in soil with MSP. The linear arrangement of data points along the diagonal identity line, crosses the 95% confidence bounds (red dashes) and confirmed by a Shapiro-Wilk test (p = 0.0012). This indicates that the data is not normally distributed.

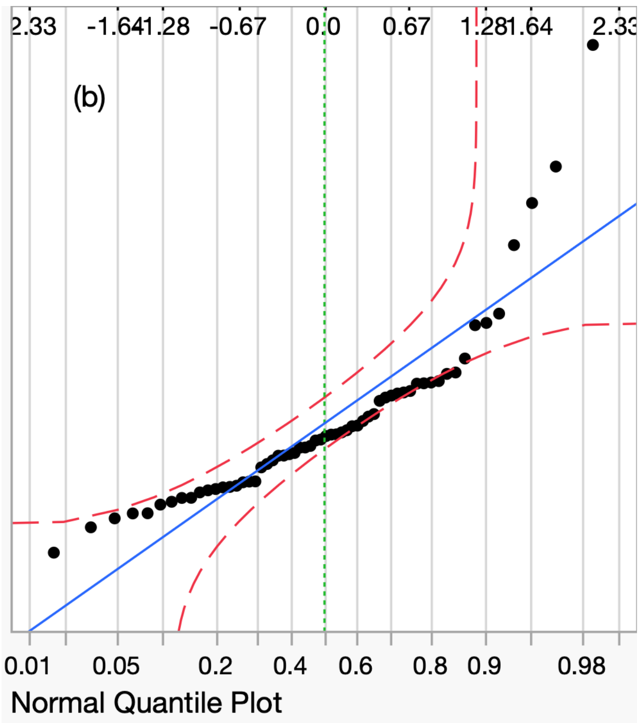

Figure A2. Q-Q plot of total biomass data or seedling sgrown in soil without MSP. The linear arrangement of data points along the diagonal identity line, crosses the 95%

confidence bounds (red dashes) and confirmed by a Shapiro-Wilk test (p = 0.0001). This indicates the data is not normally distributed.

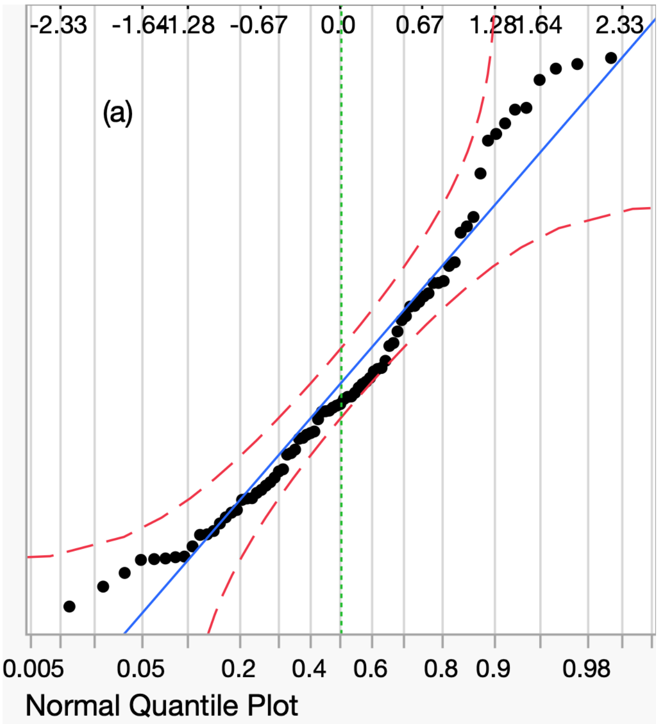

Figure A3. Q-Q plot of R:S ratio of seedlings grown in soil with MSP. The linear arrangement of data points along the diagonal identity line, crosses the 95% confidence bounds (red dashes) and confirmed by a Shapiro-Wilk test (p = 0.0012). This indicates the data is not normally distributed.

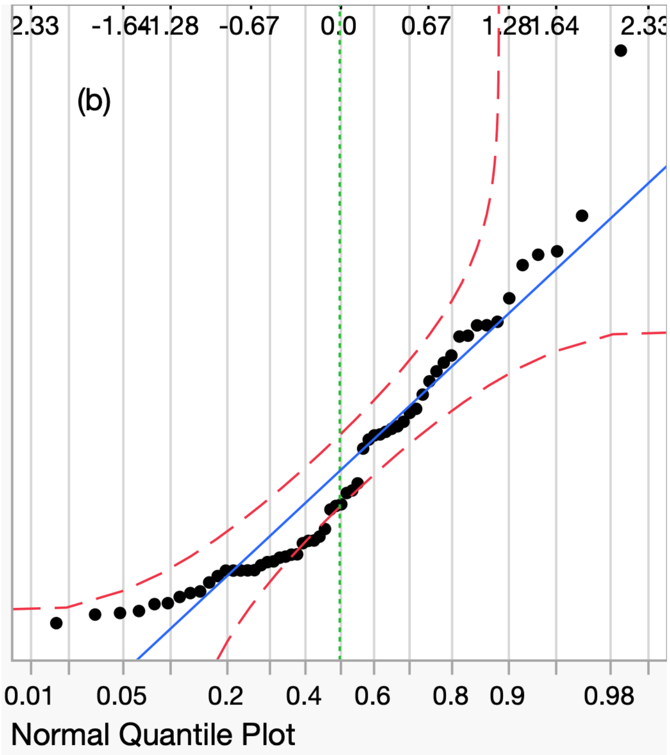

Figure A4. Q-Q plot of R:S ratio of seedlings grown in soil without MSP. The linear arrangement of data points along the diagonl identity line crosses the 95% confidence bounds (red dashes) and confirmed by a Shapiro-Wilk test (p = 0.0002). This indicates the data are not normally distributed.

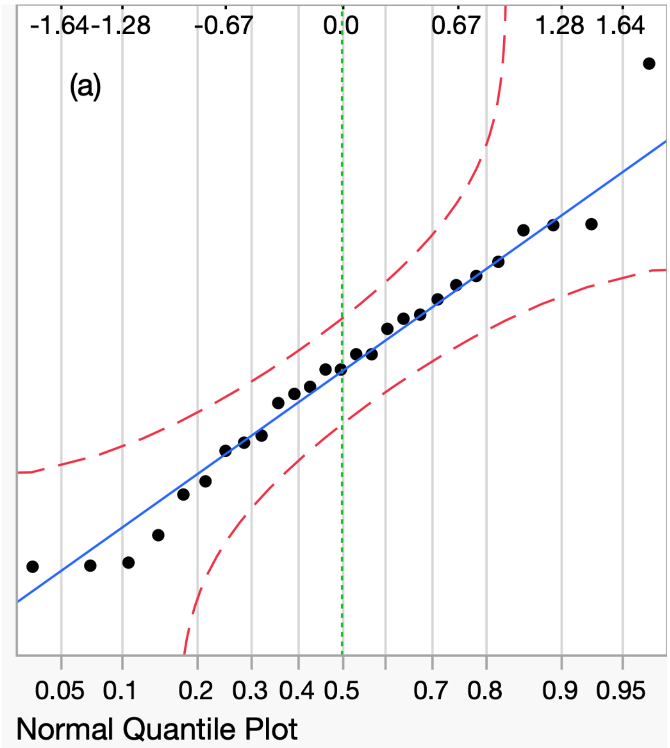

Figure A5. Q-Q plot showing the distribution of RCD from seedlings grown on soil with MSP. The linear arrangement of data points along the diagonal identity line, falling within the 95% confidence bounds (red dashes) and confirmed by a Shapiro-Wilk test (p = 0.58). This indicates the data are sufficiently normal for parametric analysis.

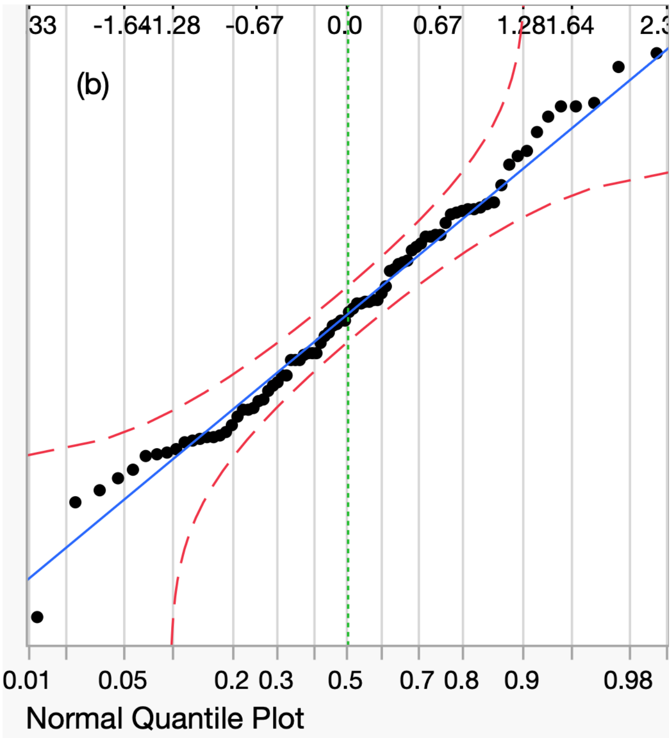

Figure A6. Q-Q plot showing the distribution of RCD from seedlings grown on soil without MSP. The linear arrangement of data points along the diagonal identity line, falling within the 95% confidence bounds (red dashes) and confirmed by a Shapiro-Wilk test ($p = 0.48$). This indicates that the data are sufficiently normal for parametric analysis.

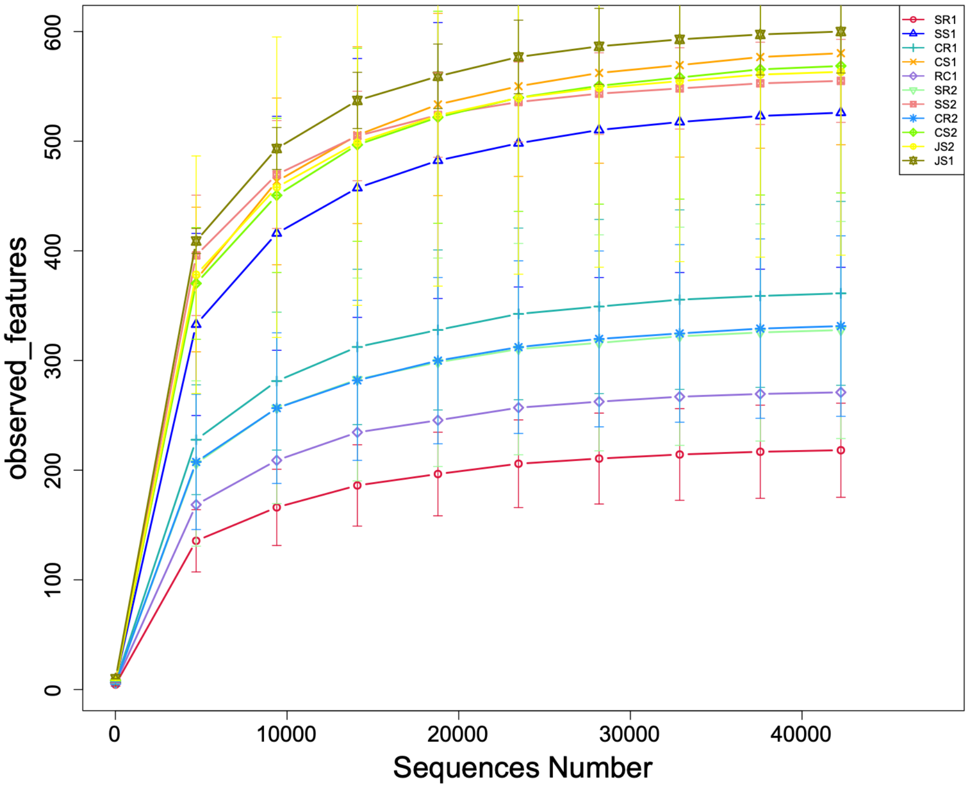

Figure A7. Rarefaction curve showing number of unique features. A steep initial increase represents high frequency of unique features, after which the curves plateaus as unique features become less frequent. Treatment nomenclature example: CS1 represents Control-Soil-MSP. Generated by Novogene.

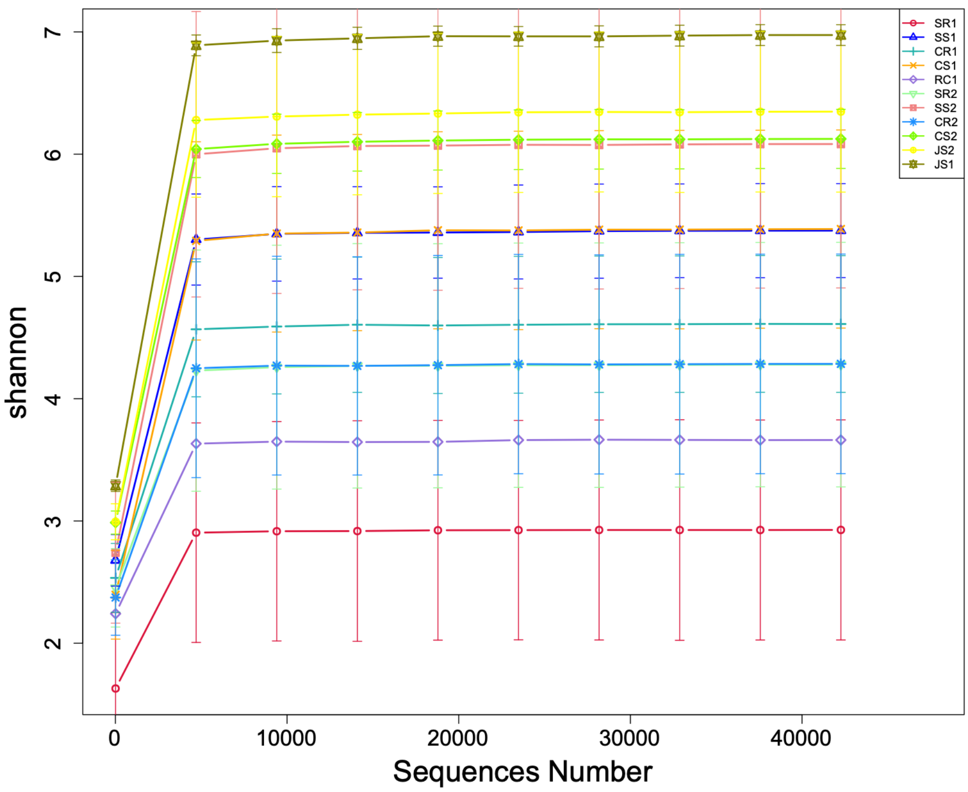

Figure A8. Rarefaction curve showing alpha diversity. The curves level off after a sharp initial increase indicating most of the diversity has been sufficiently captured. Treatment nomenclature example: CS1 represents Control-Soil-MSP. Generated by Novogene.

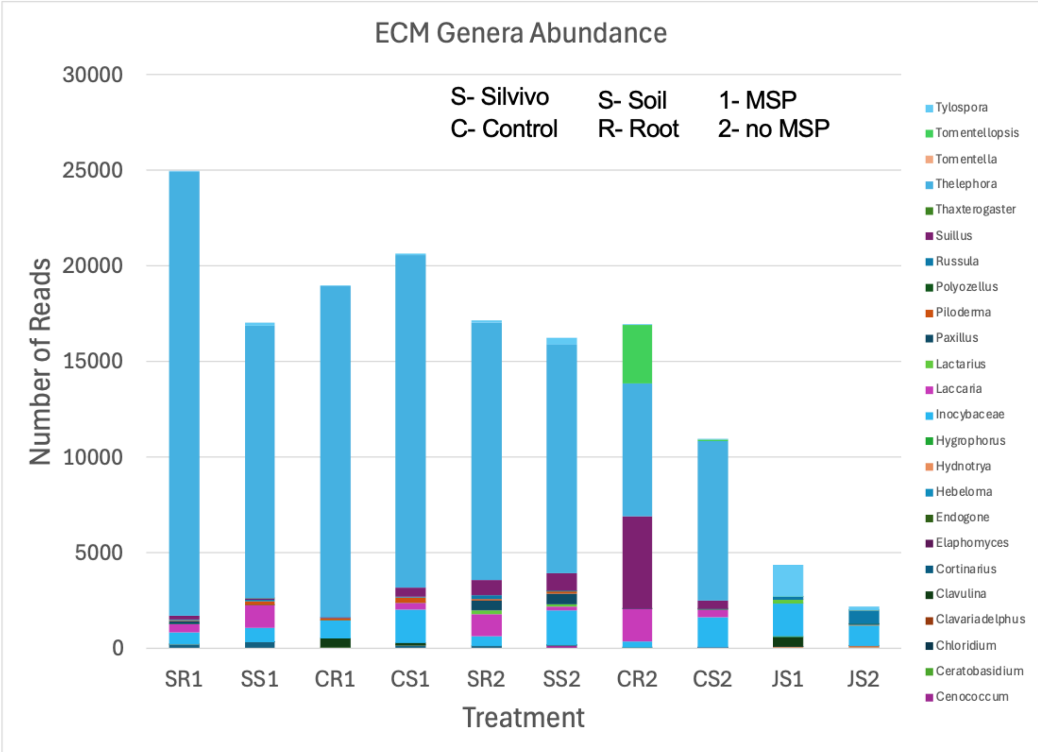

Figure A9. Bargraph of the absolute abundance of ECM genera. Treatment nomenclature example: CS1 represents Control-Soil-MSP. Adapted from Novogene.

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