# Effects of ectomycorrhizal species identity and colonisation extent on growth and nitrogen uptake in *Pinus sylvestris* seedlings

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## Abstract

Boreal forests are commonly nitrogen-limited, and Scots pine (*Pinus sylvestris)* therefore forms symbiotic associations with ectomycorrhizal fungi (EMF) to increase nutrient acquisition in exchange for photosynthates. This association is particularly important for young seedlings due to their small root systems, and most *P. sylvestris* seedlings in boreal forests are therefore highly colonised by EMF. The importance of this symbiosis for seedling growth and nitrogen uptake is usually assessed by comparing mycorrhizal and non-mycorrhizal seedlings, with less attention given to the variation in colonisation extent.

This study therefore examined whether colonisation extent can serve as a proxy for symbiotic function and predict growth and nitrogen uptake. We grew *P. sylvestris* seedlings under controlled greenhouse conditions and inoculated them with varying amounts of two EMF species, *Laccaria bicolor* and *Suillus variegatus*, to test whether inoculum dose affected colonisation. A stable isotope tracer ($^{15}N$) was applied to quantify $^{15}N$ uptake, and relationships between colonisation, growth, nitrogen content and $^{15}N$ recovery were assessed.

Both EMF successfully colonised seedlings, with colonisation increasing over time and reaching high levels after 14 weeks, independent of inoculum dose. Despite successful colonisation, mycorrhizal seedlings did not show increased growth, nitrogen content, or $^{15}N$ uptake compared with non-mycorrhizal controls, and in some cases showed reduced biomass and tracer recovery. Under these experimental conditions, mycorrhizal association therefore did not confer a growth or nitrogen benefit compared with non-mycorrhizal controls.

Fungal identity strongly influenced outcomes. Seedlings inoculated with *S. variegatus* had higher biomass, nitrogen content, and $^{15}N$ uptake than those inoculated with *L. bicolor*, despite lower levels of colonisation. Colonisation extent therefore did not predict plant performance under these experimental conditions, suggesting ectomycorrhizal effects were more strongly influenced by fungal identity.



*Keywords:* ectomycorrhizal fungi, boreal forest, seedling growth, Pinus sylvestris, nitrogen uptake, $^{15}N$ tracer, colonisation extent, greenhouse experiment

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# Abbreviations

| Abbreviation | Description |
|---|---|
| C | Carbon |
| EMF | Ectomycorrhizal fungi |
| N | Nitrogen |
| P | Phosphorus |
| RMF | Root mass fraction |

# 1. Introduction

Boreal forests are typically nitrogen-limited ecosystems due to low temperatures and slow decomposition rates (Högberg et al., 2017; Read & Perez-Moreno, 2003). Many boreal tree species therefore form symbiotic associations with ectomycorrhizal fungi (EMF) in order to improve nutrient acquisition (Jonsson et al., 1999). EMF produce extensive extraradical mycelium that can explore soil beyond root depletion zones, which can increase access to relatively immobile nutrients such as nitrogen (N) and phosphorus (P) (Finlay, 2008; Finlay & Read, 1986; Nehls et al., 2007). Some EMF are also capable of producing extracellular enzymes that mobilise nutrients from organic matter, such as leaf litter (Lindahl & Tunlid, 2015; Read & Perez-Moreno, 2003). In exchange for fungus-derived nutrients, host plants allocate a considerable proportion of their photosynthetically fixed carbon (C) compounds, primarily sugars, to the fungal symbiont (Hobbie, 2006; Nehls et al., 2010). Ectomycorrhizal colonisation is particularly important during seedling establishment, as young seedlings possess limited root systems and relatively low nutrient uptake capacity (Jones et al., 2003; Nara, 2006; Teste et al., 2009). Seedlings in naturally regenerated boreal forests are therefore often colonised by EMF within a few months of germination (Jonsson et al., 1999).

To quantify nutrient dynamics within these plant–fungal systems, a number of field and greenhouse studies have used stable isotope labelling to trace the movement of carbon and nitrogen into seedlings (Finlay & Read, 1986; Näsholm et al., 1998; Veerman et al., 2018). Stable isotope techniques exploit differences in the abundance of naturally occurring isotopic variants of elements, which differ only in neutron number and therefore have nearly identical chemical behaviour. Nitrogen, for example, occurs primarily as the lighter isotope $^{14}N$ (ca 99.63 % in atmospheric $N_2$), with a much smaller natural abundance of $^{15}N$ (0.37 atom %). By applying substrates artificially enriched in $^{15}N$ and subsequently measuring its incorporation into plant tissues, it is possible to trace nitrogen uptake and movement through plant–soil systems at both ecosystem and individual plant scales.

$^{15}N$ tracing has also been used to infer aspects of EMF functioning, as EMF can influence nitrogen partitioning within the symbiosis (Finlay et al., 1990; Pena & Polle, 2014). Generally, fungi may retain a greater proportion of acquired $^{15}N$ in fungal biomass, while transferring more $^{14}N$-enriched nitrogen to the plant host (Finlay et al., 1990; Högberg et al., 1999). However, plants may also directly acquire applied $^{15}N$ through their roots, independent of mycorrhizal associations (Finlay et al., 1988). Consequently, tracing the movement of $^{15}N$ from fungus to plant requires consideration of all components of the ectomycorrhizal system, comprising fungal and plant tissue. Furthermore, previous studies have shown that EMF can retain nitrogen within fungal biomass for variable periods before

transfer to host plants and nitrogen transfer can therefore occur with a time lag (Finlay et al., 1990; Pena et al., 2013).

More broadly, out-planting, greenhouse, and nursery studies have shown that ectomycorrhizal associations can increase seedling survival and growth (Bear & Otta, 1981; Marx & Hatchell, 1986; Rudawska et al., 2017; Vaario et al., 2008). However, these associations are not always beneficial (Johnson et al., 1997) and under conditions of high nutrient availability, ectomycorrhizal fungi may still draw substantial amounts of carbon from the host plant while providing little additional nutritional benefit beyond what the plant can acquire on its own (Karst et al., 2008; Nehls et al., 2007). In such cases, seedlings may experience reduced growth and nitrogen depletion, a pattern that can be especially pronounced in young plants (Colpaert et al., 1996; Nehls et al., 2007). Ectomycorrhizal interactions can therefore also be regarded as falling on a continuum from mutualistic to parasitic (Johnson et al., 1997; Karst et al., 2008).

As an alternative to this economic view of ectomycorrhizal associations, resource flow in EMF-plant systems can also be considered through the framework of modified source-sink dynamics, where carbon and nutrients would flow from areas of high density (sources) to areas of low density (sinks) (Bogar, 2024; Bunn et al., 2024). Carbon, for example, would flow from the plant, as a source of carbon-containing photosynthates, to the C-sink fungal mycelium, and vice versa for nutrients (Bogar, 2024). It has been observed that when plant growth is limited by environmental factors other than carbon availability, such as nutrient limitation or drought, plant photosynthesis can continue at a high rate, producing surplus photosynthates (Körner, 2003). When the plant cannot utilise these due to growth constraints belowground C fluxes can increase, particularly root exudation (Prescott et al., 2020). Root exudates are taken up by various competing sinks in the soil, including EMF, which require carbon for mycelial growth (Cairney, 2012; Corrêa et al., 2011; Pena et al., 2023; Prescott et al., 2020). A ‘carbon surplus’ framework, formulated by Prescott et al. (2020), therefore suggests that frequently, under natural conditions, the distribution of surplus carbon to the soil environment, including EMF, may explain C flow in the EMF-plant system, without necessitating intentional resource trading. Neutral or lower growth in mycorrhizal plants would be possible under a modified sink-source dynamic framework, if the EMF carbon sink is particularly strong, without necessitating parasitism as a descriptive outcome (Bogar, 2024).

Considering these various explanatory frameworks, the manner in which ectomycorrhizal effects are evaluated in experimental studies is particularly important. Ectomycorrhizal effects are commonly assessed using inoculated versus non-inoculated plants, with less attention given to whether variation in

colonisation extent itself predicts functional outcomes such as seedling growth and nitrogen acquisition (Read & Perez-Moreno, 2003). This is despite evidence that colonisation can vary substantially among individuals and treatments, and that such variation does not always translate into proportional effects on plant performance (Hobbie, 2006; Hobbie et al., 2009; Pena et al., 2013). Furthermore, while inoculum dose is often used as a proxy for expected colonisation, the relationship between dose and realised colonisation is not always consistent, suggesting that colonisation is not solely determined by inoculum input (Arnebrant & Söderström, 1992; Mortier et al., 1988; Pera et al., 1998). In addition, ectomycorrhizal fungal species may differ not only in colonisation efficiency, but also in the extent to which colonisation translates into nutrient uptake and transfer to the host (Hobbie et al., 2009; Pena et al., 2023; Pena et al., 2013). Taken together, this introduces uncertainty into how reliably commonly used experimental proxies, such as inoculum dose and colonisation proportion, reflect functional effects of ectomycorrhizal associations on plant nitrogen uptake and growth.

The aim of this study was therefore to explore whether ectomycorrhizal colonisation of *Pinus sylvestris* can be manipulated through inoculum dose, and how variation in colonisation levels influences seedling growth and $N/^{15}N$ acquisition. *Pinus sylvestris* was chosen as the plant host, as it is a dominant and widespread tree species in northern Sweden and is an important timber species. To assess species-specific EMF effects, seedlings were inoculated with either *Suillus variegatus* or *Laccaria bicolor*. Both are frequently found in association with *P. sylvestris*. Naturally regenerated *P. sylvestris* seedlings were collected from a boreal forest site in northern Sweden to provide a field context for the relationship between colonisation levels and plant $^{15}N$ and N uptake.

The following hypotheses were tested in this study:

***H1.*** Altering inoculum dose will affect colonisation levels, with higher doses leading to greater colonisation levels, although this effect will be bounded at very high percentages. *S. variegatus* will show lower colonisation levels than *L. bicolor* at each harvest due to its slower colonisation abilities.

***H2.*** Mycorrhizal seedlings will show greater growth, foliar N concentration, and $^{15}N$ uptake than non-mycorrhizal plants.

***H3.*** Seedling growth, N uptake, and $^{15}N$ uptake will be higher at greater colonisation levels, with this effect eventually plateauing at very high colonisation levels. A similar relationship between ectomycorrhizal colonisation and nitrogen uptake is expected in naturally regenerated *P. sylvestris* seedlings.

# 2. Materials and methods

## 2.1 Experimental design

### 2.1.1 Field experiment

Naturally regenerated Pinus sylvestris seedlings were sampled across two 50 x 50 m clear cuts in Åheden, Northern Sweden, in September 2025. The study site was a coniferous forest, composed of 80% *Pinus sylvestris*, 15% *Picea abies* and 5% *Betula pubescens* pre clear cutting. Tree ages ranged from 35 to 220 years. The ground vegetation consisted of Ericaceous dwarf shrubs, mainly *Vaccinium vitis-idaea* and *Vaccinium myrtillus*. The stand had last been harvested in March 2022, with site preparation following in October 2022 and replanting in 2023. Planted seedlings had been marked and were therefore excluded from sampling. 30 naturally regenerated seedlings were selected to cover a range of sizes, spanning from ca. 5-20 cm. Of these, 15 seedlings were taken from locations at least 8 m away from the base of the nearest tree to ensure they were outside of or at the very edge of the rooting zone, and a further 15 from within intact areas to ensure they fell within the rooting zone (Göttlicher et al., 2008; Henriksson et al., 2021). $^{15}N$ label solution (2.70 g of $KNO_3$ enriched to 99.8 atom % $^{15}N$ and diluted in 15 litres of water, 500 ml of solution per seedling) was applied to the soil surface surrounding seedlings two weeks prior to harvesting. Seedlings were collected with surrounding soil and vegetation to maintain intact root systems before processing in the lab.

### 2.1.2 Greenhouse experiment

*Plant materials and growth conditions*

All seedlings used in the experiment were grown from seed in late October-early November 2025. 325 seeds were first soaked for three nights and then surface-sterilised by soaking in 15% hydrogen peroxide for 20 minutes and rinsing several times with water. Sterilised seeds were germinated on water agar (1%) plates with glucose (3%), to trigger any contamination, for two weeks. Following germination, seedlings were transferred to transparent 540 ml plastic filter boxes. The boxes were each filled with 75 g of a peat/vermiculite mixture (80/20 proportion) which had been moistened to 65% dry weight with ½ MMN solution (half-strength Modified Melin-Norkrans medium), excluding glucose and agar, and autoclaved.

The filter boxes were sealed to avoid contamination with other fungal species, as frequently occurs in greenhouse studies (Browning & Whitney, 1992).

Seedling gas exchange was maintained via filter-covered openings in the container lids. All other growth conditions were also controlled, with day/night length set to 18/6 hours, temperature to 20°C/15°C, and relative humidity to 60%. Air temperature and relative humidity inside filter boxes were monitored using wireless loggers (Ruuvi Innovations Ltd (Oy)). To ensure roots were growing in darkness, the bottom half of the filter boxes was covered in aluminium foil. All seedlings were watered with 60 ml Milli-Q water at inoculation and again with 20 ml Milli-Q water twice during the growth period. Nutrients were supplied once during the experiment in the form of 20 ml ½ MMN (without glucose) given one month after inoculation.

*Fungal cultures*

Two fungal species were selected for use in the experiment: *Laccaria bicolor* and *Suillus variegatus*. Both *L. bicolor* and *S. variegatus* have previously been successfully cultivated with *Pinus sylvestris* seedlings or those of other *Pinus* species (Kasuya, 1992). Fungal mycelia were initially cultured on agar plates in September 2025 and then converted to liquid cultures in October 2025. For this, a total of 300 plugs (150 from each fungal species) of 5 mm diameter each were taken from the actively growing periphery of the mycelia and mixed with 1800 ml ½ MMN (excluding agar). Liquid cultures were then transferred to an incubator and shaken at 23°C for 3 weeks. Finally, liquid cultures were homogenised into a slush before being pipetted into the soil of the filter boxes.

*Variable inoculation treatments*

To test the effects of variable colonisation by the two different ectomycorrhizal fungal species, 210 seedlings were randomly assigned to one of five treatments (Table 1). Inoculum was applied at the same time as germinated seeds were transferred from agar plates to the filter boxes containing substrate. The prescribed inoculum volume was pipetted into the soil across several locations in the box.

***Table 1*** *Overview of ectomycorrhizal fungi inoculum dose treatments applied to Pinus sylvestris seedslings in the greenhouse study.*

| Treatment | Fungal species | Inoculum volume | Label |
|---|---|---|---|
| Control | None | 0 ml | fu0 |
| Low | *Laccaria bicolor* | 4 ml | *Lb4* |
| Low | *Suillus variegatus* | 4 ml | *Sv4* |
| High | *Laccaria bicolor* | 8 ml | *Lb8* |
| High | *Suillus variegatus* | 8 ml | *Sv8* |

*Harvest*

The experiment was initially designed to include four harvests distributed throughout the growth period. However, only 57 germinated seeds (32%) developed into seedlings, resulting in unequal replicate numbers across treatments (18 control, 12 *L. bicolor* 8 ml, 13 *L. bicolor* 4 ml, 8 *S. variegatus* 8 ml and 6 *S. variegatus* 4 ml). The number of harvests was therefore reduced from four to two. As so few *S. variegatus* seedlings were available, these were excluded from the first harvest and sampled only at the second harvest. This ensured an equal number of replicates at the second harvest and increased the number of replicates compared to splitting the *S. variegatus* seedlings across harvests (Table 2). An additional five unlabeled control seedlings were collected at the second harvest for reference measurements of natural $^{15}N$ abundance.

Two weeks prior to each harvest, 20 ml of $^{15}N$-labelled tracer solution (9.95 mg of $KNO_3$ enriched to 99.8 atom % $^{15}N$, diluted in 600 ml water) was applied to the soil surface of each filter box.

The first harvest was carried out in late January 2026 after 11 weeks of growth, and the second harvest was conducted in mid-February 2026 after 14 weeks of growth (Table 2). At both harvests, seedlings were separated into shoot and root components, with roots cleaned of soil and sand residues and then stored in 50% ethanol at 4 °C.

***Table 2*** *Number of Pinus sylvestris seedlings in each ectomycorrhizal fungi inoculum dose treatment harvested at 11 weeks and 14 weeks.*

| Treatment | 11 weeks | 14 weeks | Total |
|---|---|---|---|
| Control | 6 | 11* | 17 |
| *Laccaria bicolor* 8 ml | 6 | 6 | 12 |
| *Laccaria bicolor* 4 ml | 6 | 6 | 12 |
| *Suillus variegatus* 8 ml | 0 | 6 | 6 |
| *Suillus variegatus* 4 ml | 0 | 6 | 6 |
| **Total** | **18** | **35** | **53** |

* (including 5 seedlings collected for $^{15}N$ natural abundance measurements)

## 2.2 Measurements

### 2.2.1 ECM root tip identification

Cleaned roots from both greenhouse and field seedlings were used to establish extent of ectomycorrhizal colonisation. As the roots of greenhouse seedlings were still undeveloped, both the total number of root tips and the number of colonised root tips could be counted entirely using a dissecting microscope. Root tips were

classed as colonised if they fulfilled two of the following criteria: i) swollen, ii) free of root hairs, and iii) not transparent. Examples of roots considered uncolonised or colonised are shown below (Fig. 1). The proportion of ECM-colonised roots tips was calculated by dividing the number of colonised root tips by the total number of root tips.

Field seedling roots were considerably more developed than those of greenhouse seedlings. Ten field seedlings had intact root systems and were therefore scanned with an Epson Perfection V600 Photo Scanner (Regent Instruments Inc.) and analysed using WinRHIZO Pro analysis software (Regent Instruments Inc.). Extent of ectomycorrhizal colonisation was determined for all field seedlings by cutting roots into fragments of 1-2 cm length, placing these on a gridded petri dish with 100 intersections and using a dissecting microscope to determine the colonisation status of the root tip closest to each intersection. This directly provided the proportion of colonised root tips.

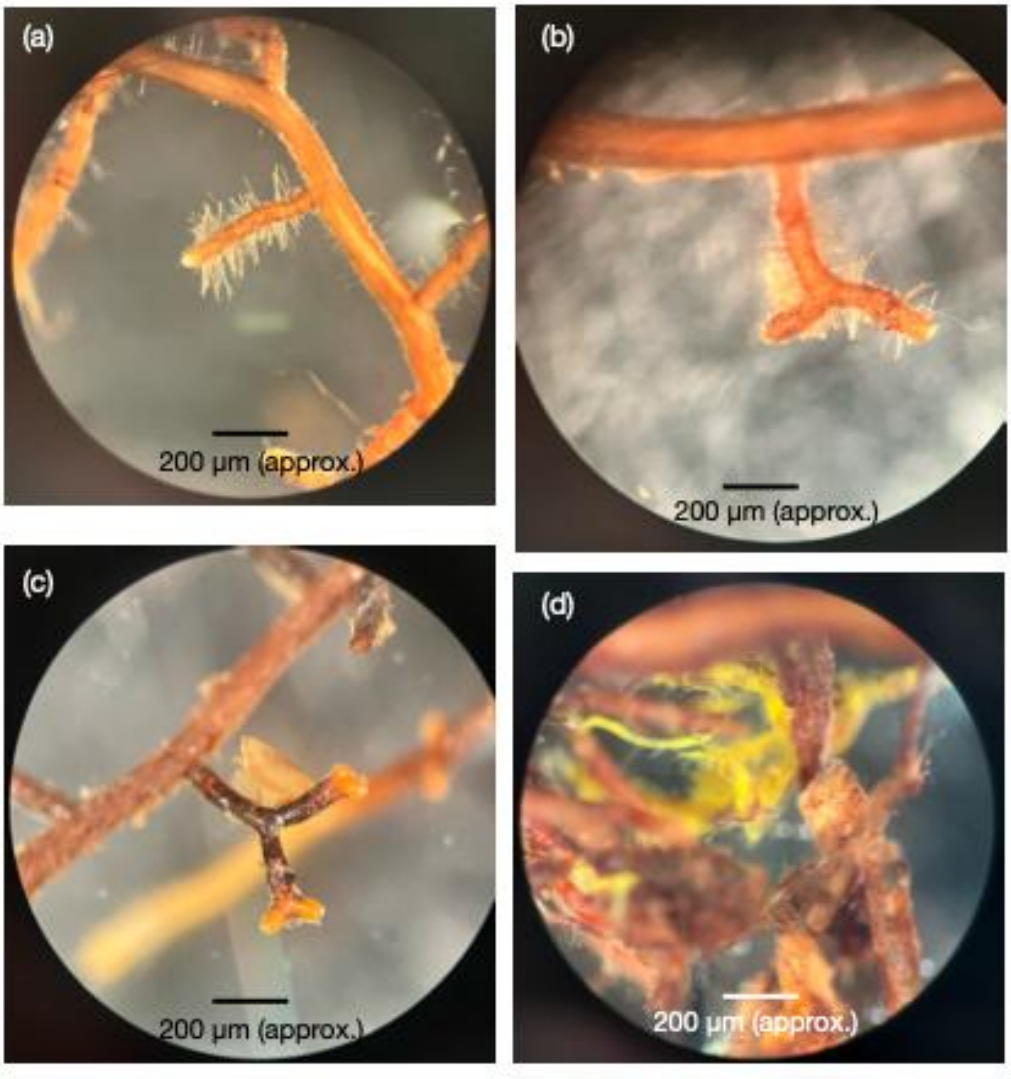


***Figure 1*** *Examples of root structure from greenhouse and field seedlings. (a) Uncolonised greenhouse root, with slender shape and root hairs, (b) Colonised greenhouse root, with bifurcated shape, darker tips but root hairs present, (c) Colonised field seedling root with bifurcation, slight swelling and dark colour, and (d) Example of ectomycorrhizal mycelium on roots of a field seedling.*

### 2.2.2 $^{15}$N uptake

Dried needle samples from all field seedlings and dried shoot samples from greenhouse seedlings were ground and 0.05 mg weighed out into uniform tin capsules for isotope ratio mass spectrometry (IRMS) analyses. For greenhouse seedlings, shoot samples were used rather than foliar ones due to their low needle dry weight. IRMS was carried out using an isotope ratio mass spectrometer (DeltaV,Thermo Fisher Scientific, Bremen, Germany) and elemental analyser (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) to determine the

mass fraction of N (ωN (%), g N per 1 g dry mass), $\delta^{15}$N (‰) and isotopic amount fraction of $^{15}$N (FN, atom %). FN was used as a measure of $^{15}$N enrichment, representing the proportion of nitrogen in the plant tissue sample present as $^{15}$N.

The fraction of applied $^{15}$N tracer recovered by each seedling, representing the proportion of the applied nitrogen taken up by the plant, was calculated using the following equation:

$$^{15}\text{N tracer recovery} = F_{sample}\left(\frac{n_{sample}}{n_{tracer}}\right)$$

where, $F_{sample}$ is the excess molar fraction of $^{15}$N in the plant tissue sample (i.e. FN corrected for the natural abundance of $^{15}$N measured in unlabelled samples), $n_{sample}$ is the total amount of N in the shoot (moles), and $n_{tracer}$ is the total amount of $^{15}$N (moles) added in the $^{15}$N tracer solution.

Four samples were excluded from any further N uptake or $^{15}$N calculations and analyses as their ωN or FN values lay outside the calibration range of the IRMS instrument.

### 2.2.3 Biomass

Both field and greenhouse seedlings were dried at 60°C for 48 hours before shoot and root dry weight were measured. The root mass fraction (RMF) was calculated by dividing dry root weight by total seedling dry weight.

## 2.3 Statistical analyses

All statistical analyses were carried out in R version 4.5.2 (R Core Team, 2025). Linear regression analyses were performed using generalised linear models (GLMs) fitted using the *glmmTMB* package version 1.1.14 (McGillycuddy, 2025). This allowed for appropriate error distributions and link functions for each response variable. Simulated residuals were generated for each model and assessed for fit against the expected distribution and against predicted values using *DHARMa* version 0.4.7 (Hartig, 2024). Significant predictors identified in the GLMs were further examined using estimated marginal means (EMMs) and pairwise comparisons in the *emmeans* package version 2.0.3 (Lenth & Piaskowski, 2026), with Tukey-adjusted *p*-values used to account for multiple comparisons. For ease of interpretation, model coefficients were exponentiated and presented as odds ratios for logit-link models and response ratios for log link models. All graphs were produced with *ggplot2* 4.0.2 (Wickham, 2016), and model summaries, including exponentiated estimates, were tabulated using *sjPlot* 2.9.0 (Lüdecke, 2025).

All field seedlings showed root colonisation greater than 90%, which was not considered sufficient variation for analyses to be run, and they were therefore

excluded from analyses. They were retained only as a reference for comparison with greenhouse seedlings.

Analyses were split into three strands: 1) inoculation protocol evaluation, 2) comparisons of growth and N/$^{15}$N uptake between mycorrhizal and non-mycorrhizal seedlings and 3) evaluation of the effect of colonisation, fungal identity and harvest on mycorrhizal seedling growth and N/$^{15}$N uptake.

### 2.3.1 Protocol evaluation

To assess the relationship between fungal inoculum dose and colonisation levels, the proportion of colonised roots was compared across treatment and control groups using beta-binomial GLMs with a logit link. Separate models were run for each harvest and a third model compared *L. bicolor* seedlings across both harvests. Contaminated controls were excluded from these models as the identity of the naturally colonizing fungus was not known, as were uncolonised seedlings, as these were non-mycorrhizal but had been exposed to EMF inoculum. Pairwise treatment comparisons were performed using estimated marginal means.

### 2.3.2 Effect of mycorrhization

To examine the overall effect of mycorrhization on growth and N/$^{15}$N uptake, control seedlings were compared to mycorrhizal seedlings (with both fungal species pooled). Seedlings from the first and second harvest were analysed in separate models to account for different developmental ages and because *S. variegatus* seedlings could not be included in the first harvest.

Each of the four N/$^{15}$N uptake and four growth measurements were used as response variables, with mycorrhization status (i.e. mycorrhizal vs. non-mycorrhizal) used as a predictor. As data were continuous and positively skewed, lognormal distributions with a log link were used for all variables except percentage tracer recovery and root mass fraction. Both of these variables were examined in models using a beta family distribution with a logit link as they are bounded between 0 and 1 (after conversion from percentages where necessary) and showed high heteroskedasticity. Inference was based on estimated marginal means and Tukey-adjusted pairwise comparisons.

### 2.3.3 Effect of colonisation levels and fungal species identity

A similar approach was used as for mycorrhization analyses; however, only mycorrhizal seedlings from the second harvest (14 weeks) were included. This approach was chosen because the second harvest allowed well replicated comparisons between both of the fungal species. Control seedlings were excluded

as previous analyses had already explored whether mycorrhization itself influenced the response variables, and this analysis therefore aimed to clarify the effects of colonisation extent and fungal species identity on response variables among mycorrhizal seedlings.

The same distributions were used as in previous analyses, with the exception of root dry weight, which was modelled using a Gamma distribution with a log link. Fungal species identity and colonisation extent were included as predictor variables in each model. Inference was based on estimated marginal means and Tukey-adjusted pairwise comparisons.

### 2.3.4 Effect of seedling age

The same approach was used as for analyses of colonisation levels and fungal species identity; however, only *L. bicolor* inoculated seedlings were included. As these were the only seedlings collected at both harvests, this approach allowed the effect of seedling age (harvest time) on each response variable to be assessed. Harvest was used as the only predictor, instead of colonisation, as colonisation levels at the first harvest were significantly lower than at the second with no overlap, and colonisation was therefore highly confounded with harvest. Inference was based on estimated marginal means and Tukey-adjusted pairwise comparisons.

# 3. Results

## 3.1 Protocol evaluation

### 3.1.1 Colonisation success

The inoculation protocol resulted in successful colonisation of most seedlings across both harvests. At the first harvest, 10 out of 12 (83%) of inoculated seedlings were colonised, of which six were *Lb4* and four were *Lb8*. At the second harvest, 23 of 24 (96%) inoculated seedlings were colonised, with all *Lb4*, *Lb8* and *Sv8* seedlings showing colonisation, and only one *Sv4* seedling showing no signs of colonisation. The two uncolonised *Lb8* seedlings from the first harvest had the lowest number of root tips, root dry weight and needle dry weight, with no consistent difference in shoot dry weight.

Natural colonisation of the non-inoculated controls occurred at both harvests. In the first harvest (11 weeks), four out of six harvested controls (67%) were colonised (2-18% colonisation). At the second harvest (14 weeks), one out of six control seedlings showed colonisation (14%). The identity of the fungus naturally colonizing the control seedlings was not known.

## 3.2 The effect of inoculum amount on colonisation

Colonisation differed between inoculated and control seedlings at both harvests, but there were no statistically significant differences among inoculation treatments. At the first harvest, higher colonisation levels were found for both *Lb4* (odds ratio = 0.25, 95% CI: 0.07-1.00, Tukey-adjusted $p = .041$) and *Lb8* (odds ratio = 0.19, 95% CI: 0.05-0.78, $p = .017$) treatments compared to the control, whereas *Lb4* and *Lb8* did not significantly differ from each other ($p = .824$; Fig. 2a). Similarly, at the second harvest significantly higher colonisation levels occurred in both *L. bicolor* and *S. variegatus* treatments compared to the control treatment (all Tukey-adjusted $p < .001$), whereas no differences were detected between inoculated treatments themselves (all $p > .05$; Fig. 2b).

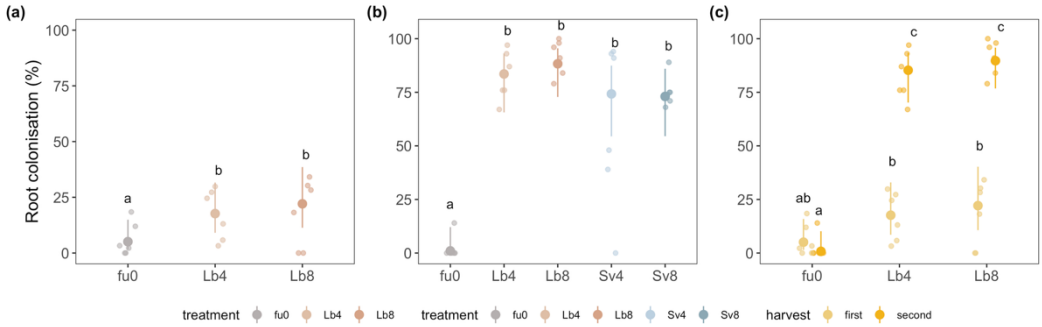


***Figure 2*** *Estimated marginal means (from generalised linear models) showing the effect of different ectomycorrhizal fungi species dose treatments compared to controls for a) the first harvest after 11 weeks, b) the second harvest after 14 weeks, and c) for Laccaria bicolor inoculated seedlings across both harvests. Raw data points are shown as individual observations, with model-estimated marginal means ± SE overlaid. Letters indicate statistically significant differences based on Tukey-adjusted pairwise comparisons of estimated marginal means. Abbreviations stand for: "fu0" = control seedlings, "Lb4" = inoculation with 4 ml of L. bicolor, "Lb8" = inoculation with 8 ml of L. bicolor, "Sv4" = inoculation with 4 ml of S. variegatus, and "Sv8" = inoculation with 8 ml of S. variegatus.*

Overall, colonisation levels increased over time. At the first harvest, colonisation levels ranged from 2-40% with a mean of 21.5% (± 10.8 SD), while at the second harvest 39-100% of roots were colonised, with a mean of 80.9% (± 15.7 SD). Pairwise comparisons showed that colonisation of *L. bicolor*-inoculated seedlings increased significantly between the first and second harvests, whereas control seedlings did not (Fig. 2c). Colonisation extent was higher in *Lb8* seedlings than controls at the first harvest (odds ratio = 0.19, $p = .049$; Fig. 2c), but did not vary significantly between *Lb4* and controls ($p > .05$; Fig. 2c). At the second harvest, both *Lb4* and *Lb8* seedlings showed significantly higher colonisation than controls (all $p < .001$; Fig. 2c). Colonisation extent also increased significantly between harvests within both inoculated treatments (all $p < .001$), while no difference was found between *Lb4* and *Lb8* at either harvest (all $p > .05$; Fig. 2c). Field-collected seedlings all showed high colonisation levels, with more than 90% of roots colonised, providing a contextual reference for the greenhouse seedlings (Fig. 3a).

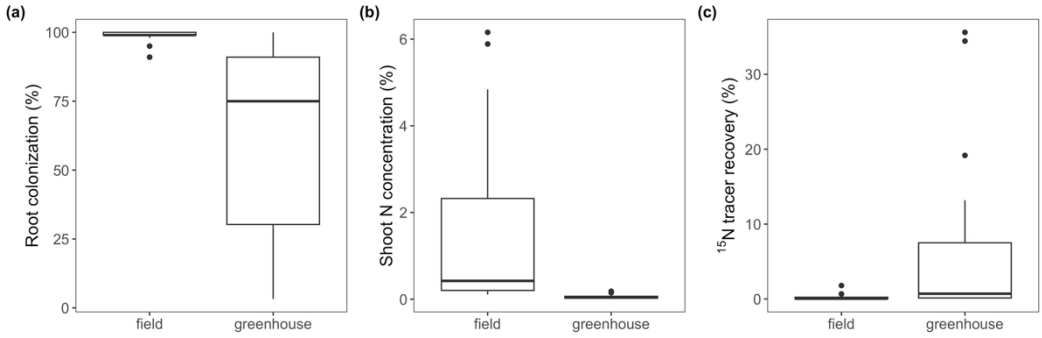


***Figure 3*** *Differences between field and greenhouse seedlings in (a) root colonisation (%), (b) shoot N concentration (%), and (c) $^{15}N$ tracer recovery (%). Field seedling values are provided as natural reference values only and were not included in analyses of seedling growth and nitrogen uptake. Greenhouse seedlings from both harvests, at 11 and 14 weeks, are included.*

## 3.3 Nitrogen uptake and growth

### 3.3.1 Effect of mycorrhization on seedling performance

Most growth variables did not vary significantly between mycorrhizal and non-mycorrhizal seedlings (Fig. 4a-d). The exceptions were total and root dry weight, where mycorrhizal seedlings from the second harvest had a lower mean values than non-mycorrhizal seedlings (total dry weight: ratio = 0.68, 95% CI: 0.48-0.97, $p$ = .032; root dry weight: ratio = 0.52, 95% CI: 0.33-0.81, $p$ = .004; Fig. 4a, b). However, this effect was not seen for the first harvest or across any of the other growth variables ($p$ > .05).

There was no significant difference in seedling nutritional status, as measured by shoot N concentration, or in shoot N content, between mycorrhizal and non-mycorrhizal seedlings at either harvest ($p$ > .05; Fig. 4e, f). Mycorrhizal seedlings were on average significantly less $^{15}N$ enriched than non-mycorrhizal seedlings at both the first harvest (ratio = 0.38, 95% CI: 0.16-0.87, $p$ = .022; Table 4, Appendix 1), and at the second (ratio = 0.33, 95% CI: 0.19-0.56, $p$ < .001; Fig. 4g). Mycorrhizal seedlings also recovered significantly less $^{15}N$ tracer at both the first harvest (odds ratio = 0.31, 95% CI: 0.10-0.98 , $p$ = .046; Fig. 4h) and second (odds ratio = 0.35, 95% CI: 0.13-0.94, $p$ = 0.037; Fig. h). Descriptive statistics for all response variables are provided in Appendix 1.

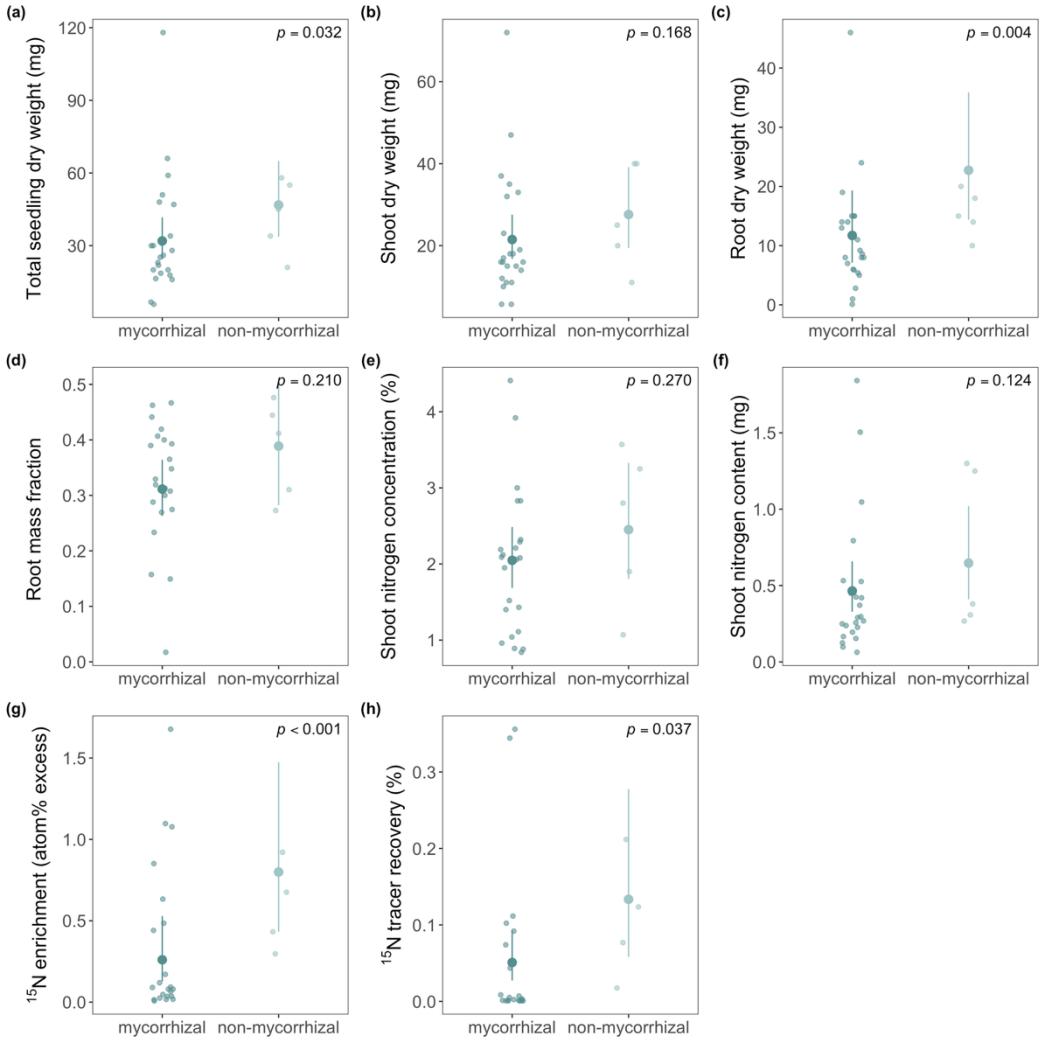


***Figure 4*** *Estimated marginal means (from generalised linear models) comparing 14-week-old mycorrhizal and non-mycorrhizal Pinus sylvestris seedlings' (a) total seedling dry weight (mg), (b) shoot dry (mg), (c) root dry weight (mg), (d) root mass fraction, (e) shoot nitrogen concentration (%), (f) shoot N content (mg), (g)* $^{15}N$ *enrichment (atom% excess), and (h)* $^{15}N$ *tracer recovery (%). Raw data points are shown as individual observations, with model-estimated marginal means ± SE overlaid.*

### 3.3.2 Effect of colonisation extent and fungal species identity on seedling performance

Seedlings inoculated with *L. bicolor* had a significantly higher proportion of colonised roots tips than those inoculated with *S. variegatus* (odds ratio = 2.10, 95% CI: 1.07-4.12, $p$ = .032; Fig. 5a). Seedlings inoculated with *L. bicolor* had lower total dry weight (odds ratio = 0.61, 95% CI: 0.42-0.87, $p$ = .007; Fig. 5b), shoot dry weight (odds ratio = 0.49, 95% CI: 0.31-0.76, $p$ = .002; Fig. 5c) and root dry weight (ratio = 0.45, 95% CI: 0.22-0.90, $p$ = .025; Fig. 5d), than seedlings

inoculated with *S. variegatus*. However, there was no significant difference in root mass fraction ($p > 0.05$; Fig. 5e).

Although shoot N concentration did not differ significantly between fungal species ($p > .05$; Fig. 5f), shoot N content was significantly lower in *L. bicolor*-inoculated seedlings (odds ratio = 0.63, 95% CI: 0.43-0.92, $p = .017$; Fig. 5g). *L. bicolor*-inoculated seedlings were also significantly less $^{15}N$ enriched (odds ratio = 0.47, 95% CI: 0.25-0.87, $p = .017$; Fig. 5h), although they did not recover more $^{15}N$ tracer ($p > .05$; Fig. 5i). There was no significant association between colonisation extent and any of the growth and N/$^{15}N$ uptake variables (all $p > 0.05$, Table 4, Appendix 1). Descriptive statistics for all response variables are provided in Appendix 1.

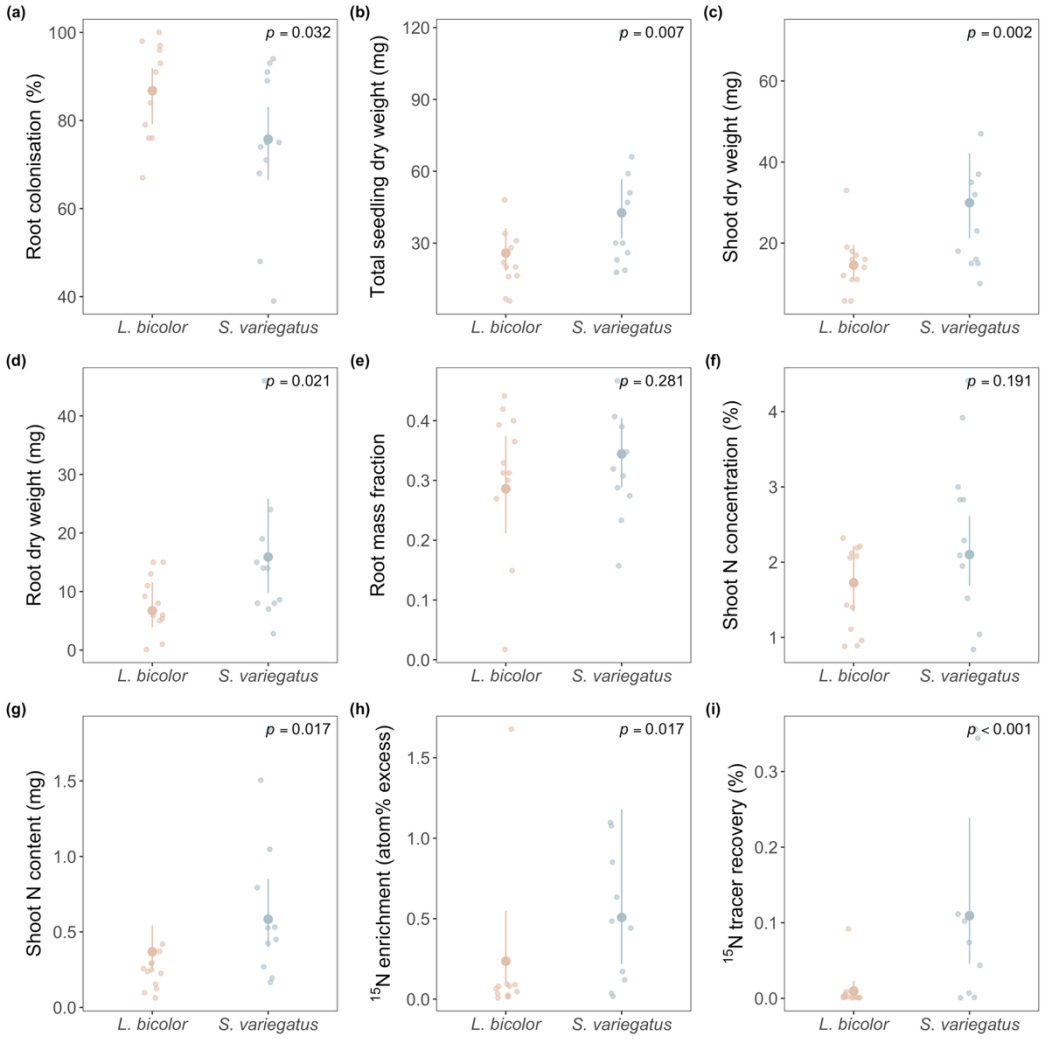


***Figure 5*** *Estimated marginal means (from generalised linear models) comparing 14-week-old mycorrhizal and non-mycorrhizal Pinus sylvestris seedlings' (a) total seedling dry weight (mg), (b) shoot dry weight (mg), (c) root dry weight (mg), (d) root mass fraction, (e) shoot nitrogen concentration (%), (f) shoot N content (mg), (g) $^{15}N$ enrichment (atom% excess), and (h) $^{15}N$ tracer recovery (%). Raw data points are shown*

*as individual observations, with model-estimated marginal means ± SE overlaid. Abbreviations stand for: "L. bicolor" = Laccaria bicolor and "S. variegatus" = Suillus variegatus.*

### 3.3.3 Effect of seedling age on seedling performance

*L. bicolor* inoculated seedlings from the first harvest had a significantly lower proportion of colonised root tips than seedlings harvested at the second (odds ratio = 0.04, 95% CI: 0.02-0.07, $p < .001$; Fig. 6a). Seedlings from the first harvest had significantly higher total dry weight (ratio = 1.78, 95% CI: 1.34-2.36, $p < .001$; Fig. 6b), shoot dry weight (ratio = 1.73, 95% CI: 1.34-2.23, $p < .001$; Fig. 6c) and root dry weight (ratio = 2.11, 95% CI: 1.35-3.50, $p = .001$; Fig. 6d) than seedlings from the second harvest. However, this was not observed for root mass fraction ($p > .05$; Fig. 6e).

Although shoot nitrogen concentration did not significantly differ between seedlings from different harvests ($p > .05$; Fig. 6f), shoot nitrogen content was significantly higher at the first harvest (ratio = 1.8, 95% CI: 1.30-2.49, $p < .001$; Fig. 6g). Similarly, while seedlings did not differ in $^{15}$N-enrichment between harvests ($p > .05$; Fig. 6h), they recovered more $^{15}$N tracer at the first harvest (odds ratio = 2.47, 95% CI: 1.12-5.45, $p = .025$; Fig. 6i). Descriptive statistics for all response variables are provided in Appendix 1.

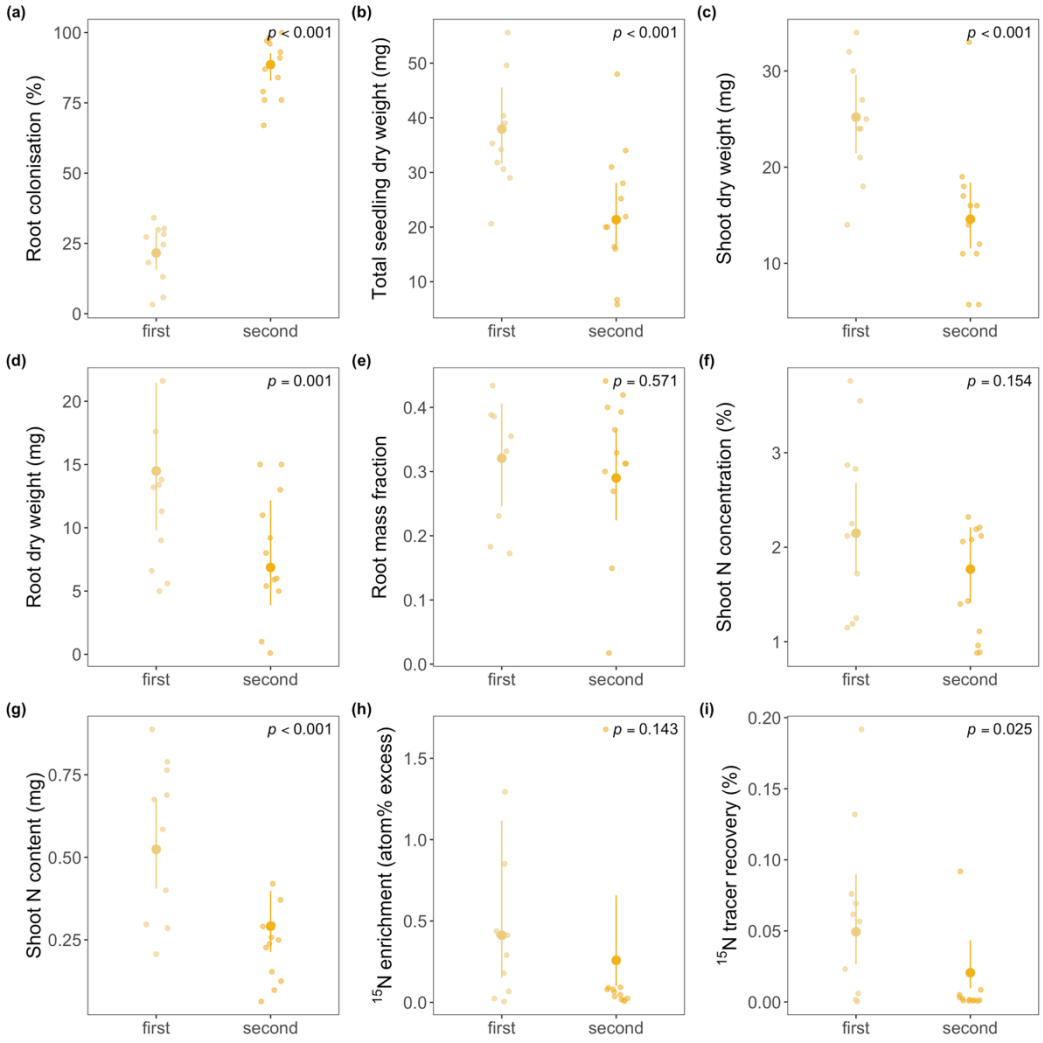


***Figure 6*** *Estimated marginal means (from generalised linear models) two harvests of Laccaria bicolor-inoculated Pinus sylvestris seedlings' (a) root colonisation (%), (b) total seedling dry weight (mg), (c) shoot dry weight (mg), (d) root dry weight (mg), (e) root mass fraction, (f) shoot N concentration (%), (g) shoot N content (mg), (h) $^{15}N$ enrichment (atom% excess), and (i) $^{15}N$ tracer recovery (%). The first harvest took place after 11 weeks and the second after 14. Raw data points are shown as individual observations, with model-estimated marginal means ± SE overlaid.*

# 4. Discussion

The inoculation protocol resulted in high levels of ectomycorrhizal root colonisation of *Pinus sylvestris* seedlings across both harvests (11 and 14 weeks after inoculation), with colonisation extent increasing significantly over time. Contrary to H1, all *Suillus variegatus* and *Laccaria bicolor* treatments achieved comparable colonisation levels, irrespective of initial inoculum amount, indicating that colonisation extent was not limited by inoculum dose under the study conditions.

Despite successful colonisation, mycorrhizal seedlings harvested after 11 weeks did not show greater growth, nitrogen (N) uptake or $^{15}N$ uptake than non-mycorrhizal seedlings, providing no support for H2 at this stage. Furthermore, mycorrhizal seedlings harvested after 14 weeks were overall smaller and exhibited reduced tracer recovery and $^{15}N$ enrichment, contradicting the expectation in H2 that mycorrhization would be associated with greater growth and N/$^{15}N$ acquisition. Treatment with different ectomycorrhizal fungi (EMF) species showed species-specific differences. Seedlings inoculated with *S. variegatus* had a lower proportion of colonised root tips but higher biomass and shoot N content, as well as greater $^{15}N$ enrichment and $^{15}N$ tracer recovery. This partially supports H1, which predicted lower colonisation by *S. variegatus*, but does not support the expectation that higher colonisation is associated with greater growth and N/$^{15}N$ uptake (H3). In contrast, within the *L. bicolor* treatment, increased colonisation after 14 weeks coincided with lower biomass, shoot N content and $^{15}N$ tracer uptake, directly opposing H3.

## 4.1 Protocol evaluation

### 4.1.1 Colonisation success

Overall, protocol application led to high rates of successful colonisation across both harvests and for all mycorrhizal treatments, with 83% of seedlings successfully colonised in the first harvest and 96% at the second. This indicates that it is well suited to inducing mycorrhization in *P. sylvestris*-*S. variegatus* and *P. sylvestris*-*L. bicolor* systems. The number of uncolonised seedlings at each harvest was very small, with two seedlings showing no evidence of colonisation at the first harvest and one seedling at the second. This has also been reported in other greenhouse studies of ectomycorrhizal associations (Browning & Whitney, 1992; Kennedy et al., 2020). Since the number of successful colonisations was otherwise high, this suggests issues with individual seedlings rather than incompatibility between host and fungal partners, as has been observed in other studies (Himanen et al., 2024; Molina, 1979). While some failed colonisation may be unavoidable, its impact was amplified in our study by high post-germination

mortality during seedling establishment, which reduced the number of replicates. Future protocol applications could potentially increase replicate retention by planting multiple seeds in each filter box and subsequently thinning to one seedling, as has been used in other greenhouse studies (Sousa et al., 2012).

In addition to seedling establishment, contamination was also present during the experiment, particularly at the first harvest, despite growing seedlings in filter boxes and only handling these or other experimental materials in laminar flow hoods to ensure sterility. This is common in ectomycorrhizal greenhouse studies generally and can be difficult to avoid entirely (Browning & Whitney, 1992; Stottlemyer et al., 2008). Complete and long-term sterility is challenging to maintain, as sterilised soils are susceptible to rapid recolonisation from surviving propagules and environmental sources during storage and incubation (Mukjang et al., 2022).

### 4.1.2 Overall colonisation levels across harvests

The proportion of colonised roots was high in our study, with extent ranging from 2-40% with a mean of 21.5% (± 10.8) at the first harvest (after 11 weeks) and 39-100% with a mean of 80.9% (± 15.7) at the second harvest (after 14 weeks). This supports findings from other studies that colonisation tends to increase over time and eventually reach high percentages, up to 100%, although this does depend on fungal species and experimental conditions (Colpaert et al., 1996; Gagnon et al., 1995; Niemi et al., 2007).

Overall colonisation extent increased significantly between harvests, indicating strong development of ectomycorrhizal associations over time. A large proportion of roots were colonised at the second harvest, which is consistent with colonisation levels observed in some other studies (Gagnon et al., 1995; Niemi et al., 2007) and higher than seen in other studies (Högberg et al., 1999). The colonisation success rate and extent seen in our study confirm that the protocol is effective at producing ectomycorrhizal colonisation under controlled conditions.

### 4.1.3 Effect of inoculum amount on colonisation extent

Despite the high colonisation levels observed, inoculum amount did not affect colonisation extent at either harvest, contradicting the first component of H1 that increasing inoculum dose would increase colonisation. However, the absence of a dose effect is consistent with the expected plateau at high colonisation levels in H1. Other studies have also shown that inoculum amount does not always predict colonisation extent (Arnebrant & Söderström, 1992; Mortier et al., 1988; Riffle & Tinus, 1982). For example, Mortier et al. (1988) found when inoculating Douglas-

fir nursery seedlings with *Laccaria lacata* that while colonisation initially increased with inoculum dose this effect plateaued eventually. Similarly, Riffle and Tinus (1982) found that varying inoculum proportion from 10 to 20% in growth substrate for *P. ponderosa* and *P. sylvestris* seedlings did not have a significant effect on colonisation extent. However, there are also studies which show an increase in the proportion of colonised roots when higher doses of inoculum are applied (Himanen et al., 2024; Tan et al., 2024). As the low (4 ml) inoculum dose led to colonisation levels that were not significantly different from the high (8 ml) inoculum dose, a much smaller dose of fungal inoculum ranging from 1-2 ml would perhaps have to be trialled to induce lower colonisation. Based on our results, another possibility is that the first harvest occurred at a time point when any effects of inoculum dose had already evened out. Harvesting at earlier time points, as we had initially planned, or even more frequently, such as every week in the first month post inoculation, could perhaps detect any effects of inoculum dose, if they are present.

## 4.2 Nitrogen uptake and growth

### 4.2.1 Effect of mycorrhization on seedling performance

Mycorrhization did not alter shoot N concentration or accumulation, nor did it affect root–shoot allocation, suggesting that overall plant nitrogen status was maintained in mycorrhizal plants relative to non-mycorrhizal ones. However, mycorrhizal seedlings were consistently less enriched in $^{15}N$ and recovered less tracer, indicating reduced uptake of labelled nitrate from the tracer pool. These findings contradict H2, which predicted enhanced N uptake and $^{15}N$ tracer recovery in mycorrhizal seedlings. This pattern suggests that ectomycorrhizal associations influenced short-term nitrogen fluxes within the plant–soil system. One possible explanation is that a proportion of the acquired $^{15}N$ was retained within ectomycorrhizal fungal tissue rather than being transferred to the plant host. Nitrogen retention within ectomycorrhizal fungi is well documented, reflecting that fungal tissues can act both as interfaces for nutrient uptake and exchange and as internal nutrient sinks (Finlay et al., 1990; Högberg et al., 1999). Both Finlay et al. (1990) and Högberg et al. (1999) showed that in systems with *P. sylvestris* and various ectomycorrhizal fungal species, $^{15}N$ enrichment was highest in fungal mycelium, intermediate in plant roots and lowest in shoots, demonstrating that nitrogen acquired by the fungal partner is not necessarily fully transferred to the host plant. Retained $^{15}N$ can, for example, be incorporated into fungal metabolic pools, including amino acid compounds such as glutamate and alanine, rather than immediately being transferred to the host plant.

In parallel, the maintenance of similar shoot N content between mycorrhizal and non-mycorrhizal plants at both harvests suggests that reduced tracer uptake did not translate into a deficit in overall nitrogen status during the experimental period. This could reflect uptake of nitrogen from alternative soil pools not captured by the tracer application, or potentially the use of nitrogen acquired before tracer application. These unlabelled sources may include organic nitrogen forms accessed via ectomycorrhizal enzymatic activity or soil nitrogen pools not directly labelled by the tracer application (Näsholm et al., 2009; Read & Perez-Moreno, 2003).

At the second harvest, non-mycorrhizal seedlings had greater total and root dry weight, although this difference was not found in shoot dry weight or RMF, suggesting that the change in overall dry weight was not driven by allocation changes. It is possible that mycorrhizal seedlings had lower total and root dry weight because of carbon allocation to ectomycorrhizal fungi. It has been estimated that approximately 5–20% of plant carbon assimilates can be transferred to EMF, and this can increase with colonisation extent (Hobbie, 2006; Leake et al., 2001; Nehls et al., 2007). High demand by EMF for carbon has been demonstrated both in culture (Fransson et al., 2007) and in microcosm or pot systems (Corrêa et al., 2006; Heinonsalo et al., 2010), and has been shown to be particularly high for newly developing mycelia (Cairney et al., 1989). This supports the interpretation that ectomycorrhizal fungi can represent a substantial carbon sink within the plant–fungus system, which may influence plant growth when belowground carbon allocation by the plant is not fully compensated by nutrient acquisition through the EMF (Johnson et al., 1997). However, carbon flow to ectomycorrhizal fungi is not necessarily a simple carbon loss, but rather part of a regulated allocation system within the plant–soil system. Carbon partitioning belowground is a dynamic process in which roots and associated symbionts function as competing sinks and allocation is determined by sink strength and physiological demand rather than simple unidirectional depletion of plant resources (Kuzyakov, 2002; Kuzyakov & Xu, 2013). Ectomycorrhizal fungi therefore act as regulated sinks whose carbon demand is integrated into these host allocation processes (Bogar, 2024; Nehls et al., 2007).

More broadly, plants often assimilate carbon in excess of immediate growth demand, particularly under nutrient limitation, meaning that belowground allocation can reflect redistribution of surplus photosynthate from the plant to the soil, rather than directly constraining growth (Bunn et al., 2024; Körner, 2003; Prescott et al., 2020). Net growth response to mycorrhization is therefore expected to vary depending on environmental and experimental conditions (Johnson et al., 1997; Karst et al., 2008). For example, Colpaert et al. (1996) observed reduced dry weight in *Pinus sylvestris* seedlings associated with ectomycorrhizal fungi under conditions of abundant nitrogen, indicating that growth reductions can

occur independently of nutrient limitation. In contrast, Corrêa et al. (2006) reported that reduced growth was primarily evident under nitrogen starvation and attributed this to limited capacity of the plant to offset fungal carbon demand through increased nutrient acquisition. Together, these studies demonstrate that the growth outcomes of carbon allocation processes are context dependent, with nutrient availability and physiological conditions playing an important role. Measurements of carbon allocation to fungal tissue, for example using $^{13}C$ isotope labelling and isotope ratio mass spectrometry of plant and fungal tissue, could be a useful addition to further explore the mechanisms leading to the reduced mycorrhizal seedling growth we observed.

### 4.2.2 Effect of colonisation extent and fungal species identity on seedling performance

When mycorrhizal seedlings were compared, *S. variegatus* inoculated seedlings had higher biomass, N uptake and $^{15}N$ uptake than *L. bicolor* inoculated seedlings, despite having a lower proportion of colonised roots. Furthermore, colonisation did not predict growth or N/$^{15}N$ uptake independently of fungal identity. This provides evidence against H3, which predicted a positive relationship between colonisation extent and seedling growth and N/$^{15}N$ uptake.Together these results suggest that colonisation extent is not a reliable proxy for N/$^{15}N$ uptake under these experimental conditions, and that a positive relationship between colonisation and growth and nutrient acquisition, as proposed in H3, may plateau at high colonisation levels. This pattern has also been observed in other experiments (Stuart et al., 2023), and suggests there is a saturating threshold above which increasing colonisation does not lead to increased nutrient acquisition (Ekblad et al., 2013; Finlay et al., 1990; Jones et al., 2009). The lowest colonisation level in the second harvest was 63% and may have been close to or over the threshold in this experimental system.

The higher nitrogen uptake observed in seedlings inoculated with *S. variegatus* compared with those inoculated with *L. bicolor* may reflect intrinsic functional differences between fungal taxa in their capacity to acquire nitrogen and transfer it to the host. It is possible for different EMF species to respond divergently to N availability (Lilleskov et al., 2011). Furthermore, *S. variegatus* is considered a pine-associated specialist (Lofgren et al., 2021), whereas *L. bicolor* is generally regarded as a generalist species (Heller et al., 2008), which may contribute to differences in nutrient exchange between host and fungus. However, there are no direct comparative studies measuring nitrogen uptake or transfer efficiency between these species under the conditions used here. Therefore, while we observed differences in $^{15}N$ uptake, we cannot resolve the underlying physiological mechanisms with the present data.

An alternative explanation is that the carbon cost to *S. variegatus* seedlings was lower as colonisation was less extensive. As mentioned previously, EMF partners can require high carbon quantities from plant partners and this can be particularly pronounced for newly forming mycelia (Ekblad et al., 2013; Leake et al., 2001). Photosynthates channelled towards the fungal partner cannot be allocated to roots or used to increase metabolism required for plant N uptake and this can have a negative effect when nutrients are abundant, such as in this study. *S. variegatus* plants may therefore have been expending less photosynthates on the symbiotic interaction and better able to slightly increase their own biomass and N uptake machinery. This could also explain the higher total dry weight but not shoot-root allocation of *S. variegatus* seedlings relative to those inoculated with *L. bicolor*. From a surplus carbon source-sink or carbon surplus perspective (Bogar, 2024; Prescott et al., 2020), these patterns may reflect strong fungal sink activity and subsequent flow of C from the plant to EMF tissue.

Dual $^{15}N$ and $^{13}C$ labelling of mycorrhizal and non-mycorrhizal seedlings inoculated with different EMF species would be a possible method to trace N and C flow through the EMF-plant system and distinguish between possible explanations for the differences we observed between fungal species. In addition, quantification of fungal biomass in the soil and fungal respiration rates could be used to assess the carbon demand placed on the seedling by the EMF.

### 4.2.3 Effect of colonisation extent and seedling age on seedling performance

Colonisation increased from 11 to 14 weeks, suggesting that the ectomycorrhizal association was continuing to establish in this time. However, total, root and shoot dry weight were significantly lower in the second harvest, as were tracer recovery and shoot N content. These findings contradict expectations from H3, and suggest a negative or saturating relationship between colonisation extent and seedling performance at 14 weeks compared to 11. The decrease in overall, root, and shoot growth was not accompanied by a decrease in shoot N concentration, suggesting that reduced growth was not primarily driven by declining nutritional status in the seedlings. As colonisation by *L. bicolor* was greater at the second harvest, one possible explanation is that a greater proportion of host photosynthate was allocated to maintaining the fungal symbiont, thereby reducing carbon available for plant biomass accumulation. Increased colonisation may therefore have imposed a higher carbon demand on the host plant without a proportional increase in nutritional transfer or growth benefit. Alternatively, increasing colonisation may have increased EMF sink strength and therefore led to greater C flow to the EMF. Whether increasing colonisation increased carbon demand by the EMF or C flow to the EMF cannot be tested with present data, as C flux between *L.*

*bicolor* and the host was not measured directly. However, both shifts in the cost–benefit balance of ectomycorrhizal symbioses under different environmental conditions (Corrêa et al., 2006; Karst et al., 2008), and high EMF C sink strength with subsequent C flow have been demonstrated (Bunn et al., 2024; Corrêa et al., 2011; Leake et al., 2001), and could apply here.

The lower $^{15}N$ tracer recovery values at the second harvest indicate that greater colonisation extent does not necessarily lead to greater $^{15}N$ acquisition by the plant. A possible explanation is that less $^{15}N$ was transferred from the fungus to the seedling shoot. This has been demonstrated in other *P. sylvestris*-EMF systems. For example, Colpaert et al. (1996) inoculated semi-hydroponically grown *P. sylvestris* seedlings with one of three different EMF and found that mycorrhizal plants had consistently lower shoot N content than non-mycorrhizal plants after five and 12 weeks and that a large proportion of acquired N had been retained in fungal mycelium or root tissues. Measurements of fungal or root N and $^{15}N$ would be required to confirm this mechanism in our study. Similarly, a sudden and significant addition of nitrogen to pine-EMF systems has been observed to lead to significant loss of N from seedlings and transfer to EMF (Smith et al., 2020). When Smith et al. (2020) grew *P. contorta* seedlings in association with *S. tomentosus* and exposed these to sudden increases in nitrogen supply, seedlings in microcosm with intact hyphae lost around 60% of N, unlike seedlings in microcosms with severed hyphae. As they acknowledge this does not provide direct evidence of nitrogen transfer to the host, however, the dependence of this effect on intact hyphae strongly supports this hypothesis. Taken together these studies support the possibility that shoot N reduction in our study was due to transfer to or retention by the fungal partner species.

## 4.3 Conclusions and broader implications

The inoculation protocol can be used to successfully induce colonisation and produce a colonisation gradient in *P. sylvestris* seedlings using *S. variegatus* and *L. bicolor*. Using inoculum doses of 4 ml and 8nml does not appear to alter colonisation extent significantly for either of the two ectomycorrhizal fungi. Achieving this would therefore likely require the low inoculum dose to be even lower than 4 ml, potentially between 1-2 ml.

Mycorrhization did not confer a growth benefit in this study and was even associated with lower overall growth and $^{15}N$ uptake and enrichment at 14 weeks. Similar results were observed for 14-week old compared to 11-week old *L. bicolor* seedlings. This supports previous observations that ectomycorrhizal associations do not always have a straightforward positive effect on young seedlings. Extending the study to include older seedlings would provide an

interesting avenue for tracing the effect of ectomycorrhizal association across a range of seedling ages.

Further research using dual $^{15}N$ and $^{13}C$ labelling and tracing through the entire plant-EMF system could be used to clarify how these growth outcomes relate to N and C flows through the system. Measurements of photosynthetic rates and $CO_2$ enrichment could be useful in untangling whether seedlings grown under conditions used in this study (i.e. without nutrient or water limitation) produced surplus carbon and how this affected nutrient uptake and growth. These extensions would be particularly valuable under an experimental setup that also tests nutrient limitation. This could elucidate how effects of the mycorrhizal association vary when nutrients become limiting. As boreal forests are naturally depleted in nitrogen this could provide particularly valuable information on how effects seen in greenhouse studies are likely to translate to natural settings such as those encountered in forestry and forest restoration.

When growth and $N/^{15}N$ uptake were evaluated against colonisation and fungal species identity amongst mycorrhizal seedlings, colonisation did not predict growth or $N/^{15}N$ uptake outcomes, while fungal species identity was associated with significant differences. These results support findings from other studies that colonisation extent is not necessarily a useful proxy for functional efficiency of the symbiosis. This is further supported by our finding that *S. variegatus* seedlings showed greater N and $^{15}N$ acquisition than *L. bicolor* seedlings despite having a lower proportion of colonised root tips. The mechanisms underpinning this cannot be explained using the present data, but this would be an interesting avenue for future research.

As EMF are increasingly acknowledged to play an important role in seedling regeneration in both commercial forestry settings and restoration of forests on disturbed sites, such as former oil sands, greater understanding of how different EMF species associate with pine seedlings and when this produces plant host benefits is of importance. While using individual EMF species in a controlled greenhouse setting does not replicate natural field conditions, where EMF species associate with hosts in communities, it has the advantage of enabling isolation of the effect of individual EMF across a range of time points. This could also valuably be extended to combinations of EMF species, for example, by combining *S. variegatus* and *L. bicolor*, using additional *Suillus* species or including species such as *Paxilllus involutus*, *Hebelome cavipes* and *Cortinariuss* spp., all of which have been found to associate with *P. sylvestris* in boreal forests in northern Sweden.

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# Popular science summary

In northern forests, trees often grow in soils that are low in nitrogen, so they depend on a particular type of soil fungi, called ectomycorrhizal fungi, to access enough nutrients. This is particularly important for young seedlings, as they have small root systems and cannot explore as much soil area for nutrients. To explore the effect of these ectomycorrhizal fungi on seedlings this study looked at young Scots pine (*Pinus sylvestris*) seedlings and how they interact with two types of ectomycorrhizal fungi, *Suillus variegatus* and *Laccaria bicolor*. These fungi colonise the roots of seedlings and will provide them with nitrogen in return for carbon assimilated by the plant.

We tested whether adding more fungal inoculum would lead to more root colonisation, and whether having higher root fungal colonisation would improve plant growth and nitrogen uptake. We also used a naturally rare form of nitrogen ($^{15}N$) to trace how much nitrogen the plants actually absorbed into their shoots and needles.

The fungi successfully colonised most seedlings' roots, and colonisation increased over time. However, adding more inoculum did not increase how many of the roots were colonised. Surprisingly, association with ectomycorrhizal fungi did not consistently improve plant growth or nitrogen uptake compared with plants without fungi. In some cases, mycorrhizal plants even grew less and took up less of the tracer nitrogen. This could be because the seedlings already had access to sufficient nutrients and were therefore allocating carbon to the fungus despite not receiving much nutritional benefit from the association.

We also found that the two fungi had different effects on the seedlings. Seedlings with *Suillus variegatus* grew more and took up more nitrogen than those with *Laccaria bicolor*, even though they had less root colonisation. Overall, the results suggest that how much the fungi benefit the plant depends more on the fungal species itself than on how much of the root is colonised. These differences between fungal species could be important in forestry and forest restoration, where ectomycorrhizal fungi are sometimes applied to young seedlings to improve their survival and growth.

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# Appendix 1

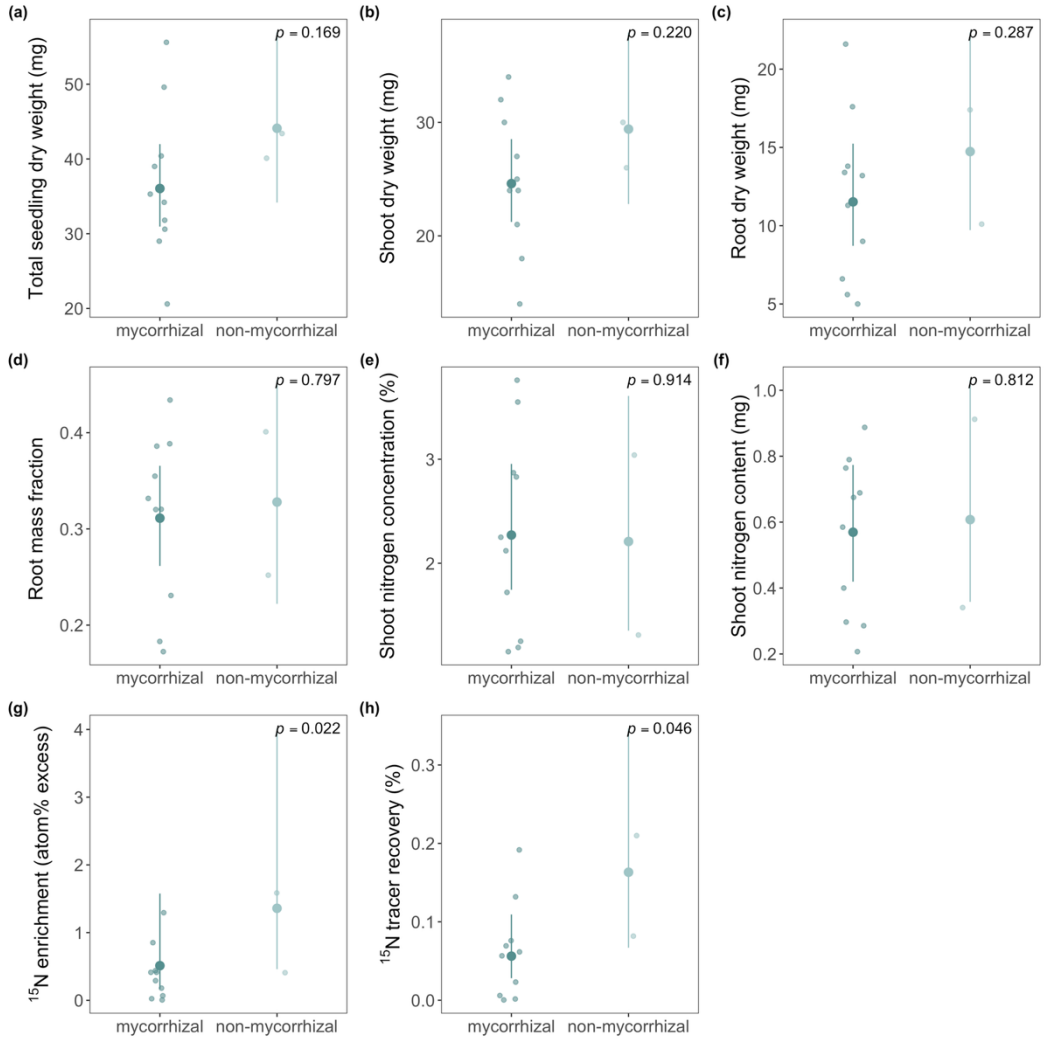


***Figure 7*** *Estimated marginal means (from generalised linear models) comparing 11-week-old mycorrhizal and non-mycorrhizal Pinus sylvestris seedlings' (a) total seedling dry weight (mg), (b) shoot dry (mg), (c) root dry weight (mg), (d) root mass fraction, (e) shoot nitrogen concentration (%), (f) shoot N content (mg), (g) $^{15}N$ enrichment (atom% excess), and (h) $^{15}N$ tracer recovery (%). Raw data points are shown as individual observations, with model-estimated marginal means ± SE overlaid.*

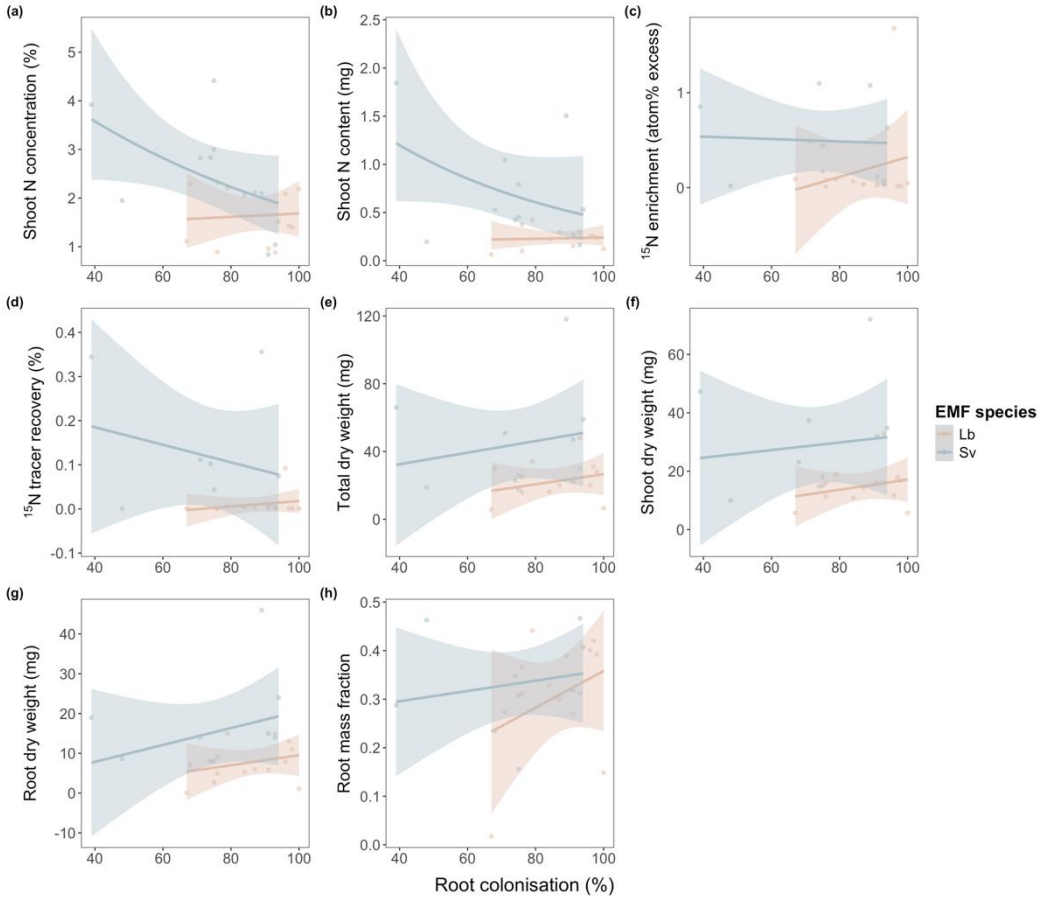


***Figure 8*** *Relationship between root colonisation (%) and N/$^{15}$N uptake and growth in 14-week-old Pinus sylvestris seedlings inoculated with either Laccaria bicolor (Lb) or Suillus variegatus (Sv). Shown are: (a) shoot N concentration (%), (b) shoot N content (mg), (c) $^{15}$N enrichment (atom% excess), (d) 15N tracer recovery, (e) total dry weight (mg), (f) shoot dry weight (mg), (g) root dry weight (mg), and (h) root mass fraction. Raw data points are shown as dots and an ordinary least squares regression line (geom_smooth with method = "lm" in ggplot2 version 4.0.2).*

***Table 3*** *Output from generalised linear models (GLMs) evaluating differences in root colonisation between different ectomycorrhizal inoculum dose treatments of greenhouse-grown Pinus sylvestris seedlings. Two harvests were carried out (11 and 14 weeks) and analysed in separate models. A third model analysed seedlings inoculated with the ectomycorrhizal fungus Laccaria bicolor across both harvests. Control seedlins were the model reference value. Abbreviations stand for "Lb4" = inoculation with 4 ml of Laccaria bicolor, "Lb8" = inoculation with 8 ml of L. bicolor, "Sv4" = inoculation with 4 ml of Suillus variegatus, and "Sv8" = inoculation with 8 ml of S. variegatus.*

| **Harvest** | **Response** | **Predictors** | **Odds Ratios** | **CI** | ***p*** |
|---|---|---|---|---|---|
| first | Root colonization | Intercept | 0.05 | 0.02 – 0.14 | **<0.001** |
| | | treatment [Lb4] | 4.01 | 1.30 – 12.32 | **0.015** |
| | | treatment [Lb8] | 5.29 | 1.61 – 17.36 | **0.006** |
| | | Observations | 18 | | |
| second | | Intercept | 0.01 | 0.00 – 0.07 | **<0.001** |
| | | treatment [Lb4] | 513 | 58.48 – 4500.06 | **<0.001** |
| | | treatment [Lb8] | 763.22 | 85.30 – 6829.13 | **<0.001** |
| | | treatment [Sv4] | 291.45 | 34.16 – 2486.41 | **<0.001** |
| | | treatment [Sv8] | 273.95 | 33.11 – 2266.64 | **<0.001** |
| | | Observations | 30 | | |
| *L. bicolor* | | Intercept | 0.05 | 0.02 – 0.14 | **<0.001** |
| | | treatment [Lb4] | 4.03 | 1.33 – 12.22 | **0.014** |
| | | treatment [Lb8] | 5.34 | 1.69 – 16.85 | **0.004** |
| | | harvest [second] | 0.15 | 0.02 – 1.28 | 0.083 |
| | | treatment [Lb4] × harvest [second] | 184.23 | 17.26 – 1966.86 | **<0.001** |
| | | treatment [Lb8] × harvest [second] | 209.48 | 19.50 – 2250.03 | **<0.001** |
| | | Observations | 36 | | |

***Table 4*** *Output from generalised linear models (GLMs) evaluating differences in N uptake, $^{15}N$ uptake and growth between ectomycorrhizal and non-mycorrhizal Pinus sylvestris seedlings harvested after either 11 or 14 weeks. Harvests were analysed separately.*

| Harvest | Response | Predictors | Estimates | CI | *p* |
|---|---|---|---|---|---|
| first | shoot N concentration | Intercept | 0.02 | 0.02 – 0.03 | **<0.001** |
| | | mycorrhizal | 0.97 | 0.59 – 1.61 | 0.914 |
| | | Observations | 12 | | |
| | | $R^2$ / $R^2$ adjusted | 0.001 / -0.221 | | |
| second | | Intercept | 0.02 | 0.02 – 0.02 | **<0.001** |
| | | mycorrhizal | 1.2 | 0.87 – 1.65 | 0.27 |
| | | Observations | 28 | | |
| | | $R^2$ / $R^2$ adjusted | 0.027 / -0.051 | | |
| first | shoot N content | Intercept | 0 | 0.00 – 0.00 | **<0.001** |
| | | mycorrhizal | 1.07 | 0.62 – 1.82 | 0.812 |
| | | Observations | 12 | | |
| | | $R^2$ / $R^2$ adjusted | 0.003 / -0.218 | | |
| second | | Intercept | 0 | 0.00 – 0.00 | **<0.001** |
| | | mycorrhizal | 1.39 | 0.91 – 2.12 | 0.124 |
| | | Observations | 28 | | |
| | | $R^2$ / $R^2$ adjusted | 0.024 / -0.054 | | |
| first | $^{15}N$ enrichment | Intercept | 0.51 | 0.17 – 1.58 | 0.243 |
| | | mycorrhizal | 2.65 | 1.15 – 6.11 | **0.022** |
| | | Observations | 12 | | |
| | | $R^2$ / $R^2$ adjusted | 0.318 / 0.167 | | |
| second | | Intercept | 0.26 | 0.13 – 0.53 | **<0.001** |
| | | mycorrhizal | 3.07 | 1.78 – 5.28 | **<0.001** |
| | | Observations | 26 | | |
| | | $R^2$ / $R^2$ adjusted | 0.164 / 0.092 | | |
| first | $^{15}N$ tracer recovery | Intercept | 0.06 | 0.03 – 0.12 | **<0.001** |
| | | mycorrhizal | 3.28 | 1.02 – 10.49 | **0.046** |
| | | Observations | 12 | | |
| | | $R^2$ | 0.122 | | |
| second | | Intercept | 0.05 | 0.03 – 0.10 | **<0.001** |
| | | mycorrhizal | 2.87 | 1.07 – 7.74 | **0.037** |
| | | Observations | 25 | | |
| | | $R^2$ | 0.151 | | |

| | | | | | |
|---|---|---|---|---|---|
| first | total dry weight | Intercept | 0.04 | 0.03 – 0.04 | **<0.001** |
| | | mycorrhizal | 1.22 | 0.92 – 1.63 | 0.169 |
| | | Observations | 12 | | |
| | | $R^2$ / $R^2$ adjusted | 0.102 / -0.097 | | |
| second | | Intercept | 0.03 | 0.02 – 0.04 | **<0.001** |
| | | mycorrhizal | 1.46 | 1.03 – 2.07 | **0.032** |
| | | Observations | 28 | | |
| | | $R^2$ / $R^2$ adjusted | 0.061 / -0.014 | | |
| first | shoot dry weight | Intercept | 0.02 | 0.02 – 0.03 | **<0.001** |
| | | mycorrhizal | 1.2 | 0.90 – 1.59 | 0.22 |
| | | Observations | 12 | | |
| | | $R^2$ / $R^2$ adjusted | 0.097 / -0.104 | | |
| second | | Intercept | 0.02 | 0.02 – 0.03 | **<0.001** |
| | | mycorrhizal [nonmyc] | 1.28 | 0.90 – 1.83 | 0.168 |
| | | Observations | 28 | | |
| | | $R^2$ / $R^2$ adjusted | 0.026 / -0.052 | | |
| first | root dry weight | Intercept | 0.01 | 0.01 – 0.02 | **<0.001** |
| | | mycorrhizal | 1.28 | 0.81 – 2.01 | 0.287 |
| | | Observations | 12 | | |
| | | $R^2$ / $R^2$ adjusted | 0.057 / -0.153 | | |
| second | | Intercept | 0.01 | 0.01 – 0.02 | **<0.001** |
| | | mycorrhizal | 1.94 | 1.24 – 3.04 | **0.004** |
| | | Observations | 28 | | |
| | | $R^2$ / $R^2$ adjusted | 0.177 / 0.111 | | |
| first | root mass fraction | Intercept | 0.45 | 0.35 – 0.58 | **<0.001** |
| | | mycorrhizal | 1.08 | 0.60 – 1.94 | 0.797 |
| | | Observations | 12 | | |
| | | $R^2$ | 0.005 | | |
| second | | Intercept | 0.45 | 0.36 – 0.57 | **<0.001** |
| | | mycorrhizal | 1.41 | 0.82 – 2.40 | 0.21 |
| | | Observations | 28 | | |
| | | $R^2$ | 0.037 | | |

***Table 5*** *Output from generalised linear models (GLMs) evaluating differences in root colonisation, growth and N/$^{15}$N uptake in Pinus sylvestris seedlings inoculated with one of the ectomycorrhizal fungi species, Suillus variegatus or Laccaria bicolor, and harvested after 14 weeks.*

| **Response** | **Predictors** | **Estimates** | **CI** | ***p*** |
|---|---|---|---|---|
| root colonisation | Intercept | 6.55 | 3.80 – 11.29 | **<0.001** |
| | fungus [Sv] | 0.48 | 0.24 – 0.94 | **0.032** |
| | Observations | 23 | | |
| shoot N concentration | Intercept | 0.02 | 0.01 – 0.03 | **<0.001** |
| | colonisation | 1.15 | 0.57 – 2.30 | 0.695 |
| | fungus [Sv] | 2.19 | 0.94 – 5.12 | 0.07 |
| | colonisation × fungus [Sv] | 0.54 | 0.26 – 1.15 | 0.113 |
| | Observations | 23 | | |
| | $R^2$ / $R^2$ adjusted | 0.281 / 0.122 | | |
| shoot N content | Intercept | 0.00 | 0.00 – 0.00 | **<0.001** |
| | colonisation | 1.06 | 0.66 – 1.68 | 0.820 |
| | fungus [Sv] | 1.59 | 1.09 – 2.31 | 0.017 |
| | Observations | 23 | | |
| | $R^2$ / $R^2$ adjusted | 0.065 / -0.083 | | |
| $^{15}$N enrichment | Intercept | 0.17 | 0.03 – 1.00 | 0.05 |
| | prop | 1.5 | 0.26 – 8.60 | 0.646 |
| | fungus [Sv] | 2.15 | 1.15 – 4.03 | **0.017** |
| | Observations | 22 | | |
| | $R^2$ / $R^2$ adjusted | 0.083 / -0.069 | | |
| $^{15}$N tracer recovery | Intercept | 0.01 | 0.00 – 0.08 | **<0.001** |
| | fungus [Sv] | 18.7 | 1.29 – 271.42 | **0.032** |
| | colonisation | 1.3 | 0.20 – 8.57 | 0.785 |
| | fungus [Sv] × colonisation | 0.66 | 0.07 – 6.54 | 0.726 |
| | Intercept | 44.38 | 15.47 – 127.33 | **<0.001** |
| | fungus [Sv] | 0.09 | 0.02 – 0.41 | **0.002** |
| | Observations | 21 | | |
| | $R^2$ | 0.36 | | |
| total dry weight | Intercept | 0.02 | 0.01 – 0.04 | **<0.001** |
| | colonisation | 1.34 | 0.81 – 2.22 | 0.248 |
| | fungus [Sv] | 1.65 | 1.15 – 2.38 | **0.007** |
| | Observations | 23 | | |
| | $R^2$ / $R^2$ adjusted | 0.124 / -0.014 | | |
| shoot dry weight | Intercept | 0.01 | 0.01 – 0.02 | **<0.001** |
| | fungus [Sv] | 2.06 | 1.31 – 3.25 | **0.002** |
| | prop | 1.24 | 0.82 – 1.89 | 0.308 |

| | | | | |
|---|---|---|---|---|
| | Intercept | 0.01 | 0.00 – 0.01 | **<0.001** |
| | fungus [Sv] | 2.28 | 0.92 – 5.69 | 0.076 |
| | Observations | 23 | | |
| | $R^2$ / $R^2$ adjusted | 0.237 / 0.068 | | |
| root dry weight | Intercept | 0.00 | 0.00 – 0.01 | **<0.001** |
| | colonisation | 1.64 | 0.74 – 3.60 | 0.221 |
| | fungus [Sv] | 2.25 | 1.11 – 4.55 | **0.025** |
| | Observations | 23 | | |
| | $R^2$ Nagelkerke | 0.238 | | |
| root mass fraction | Intercept | 0.28 | 0.14 – 0.59 | **0.001** |
| | colonisation | 1.43 | 0.82 – 2.50 | 0.208 |
| | fungus [Sv] | 1.31 | 0.80 – 2.14 | 0.281 |
| | Intercept | 8.91 | 4.07 – 19.51 | **<0.001** |
| | fungus [Sv] | 2.87 | 0.89 – 9.25 | 0.077 |
| | Observations | 23 | | |
| | $R^2$ | 0.108 | | |

***Table 6*** *Output from generalised linear models (GLMs) assessing differences in root colonisation, N/$^{15}$N uptake and growth in Pinus sylvestris seedlings inoculated with the ectomycorrhizal fungus Laccaria bicolor and harvested after either 11 or 14 weeks.*

| **Response** | **Predictors** | **Estimates** | **CI** | ***p*** |
|---|---|---|---|---|
| root colonisation | Intercept | 0.28 | 0.19 – 0.41 | **<0.001** |
| | harvest [second] | 28.18 | 15.10 – 52.60 | **<0.001** |
| | Observations | 22 | | |
| Shoot N concentration | Intercept | 0.02 | 0.02 – 0.03 | **<0.001** |
| | harvest [second] | 0.82 | 0.63 – 1.08 | 0.154 |
| | Observations | 22 | | |
| | $R^2$ / $R^2$ adjusted | 0.060 / -0.039 | | |
| Shoot N content | Intercept | 0 | 0.00 – 0.00 | **<0.001** |
| | harvest [second] | 0.56 | 0.40 – 0.77 | **<0.001** |
| | Observations | 22 | | |
| | $R^2$ / $R^2$ adjusted | 0.295 / 0.221 | | |
| $^{15}$N enrichment | Intercept | 0.41 | 0.15 – 1.12 | 0.081 |
| | harvest [second] | 0.63 | 0.34 – 1.17 | 0.143 |
| | Observations | 22 | | |
| | $R^2$ / $R^2$ adjusted | 0.031 / -0.071 | | |
| $^{15}$N tracer recovery | Intercept | 0.05 | 0.03 – 0.10 | **<0.001** |
| | harvest [second] | 0.4 | 0.18 – 0.89 | **0.025** |
| | Observations | 22 | | |
| | $R^2$ | 0.315 | | |
| total dry weight | Intercept | 0.04 | 0.03 – 0.05 | **<0.001** |
| | harvest [second] | 0.56 | 0.42 – 0.75 | **<0.001** |
| | Observations | 22 | | |
| | $R^2$ / $R^2$ adjusted | 0.377 / 0.311 | | |
| shoot dry weight | Intercept | 0.03 | 0.02 – 0.03 | **<0.001** |
| | harvest [second] | 0.58 | 0.45 – 0.74 | **<0.001** |
| | Observations | 22 | | |
| | $R^2$ / $R^2$ adjusted | 0.401 / 0.338 | | |
| root dry weight | Intercept | 0.01 | 0.01 – 0.02 | **<0.001** |
| | harvest [second] | 0.47 | 0.30 – 0.74 | **0.001** |
| | Observations | 22 | | |

| | | | | |
|---|---|---|---|---|
| | $R^2$ / $R^2$ adjusted | 0.340 / 0.270 | | |
| root mass fraction | Intercept | 0.47 | 0.33 – 0.68 | **<0.001** |
| | harvest [second] | 0.86 | 0.52 – 1.43 | 0.571 |
| | Observations | 22 | | |
| | $R^2$ | 0.011 | | |

***Table 7*** *Root colonisation extent, growth and N/$^{15}$N uptake measurements for 30 naturally regenerated Pinus sylvestris seedlings collected from a clear cut field site in northern Sweden.*

| Variable | Mean ± SD | Range | Median | IQR |
|---|---|---|---|---|
| Root colonisation (%) | 98.9 ± 1.8 | 91 – 100 | 99 | 1 |
| Total dry weight (g) | 2.042 ± 1.847 | 0.264 – 7.017 | 1.444 | 2.416 |
| Shoot dry weight (g) | 1.283 ± 1.302 | 0.212 – 4.795 | 0.725 | 1.134 |
| Root dry weight (g) | 0.758 ± 0.988 | 0.051 – 4.505 | 0.33 | 0.583 |
| Root mass fraction | 0.35 ± 0.20 | 0.11 – 0.87 | 0.29 | 0.28 |
| Shoot N concentration (%) | 1.74 ± 0.60 | 0.80 – 2.72 | 1.65 | 1.08 |
| Shoot N content (mg) | 0.015 ± 0.019 | 0.001 – 0.062 | 0.004 | 0.021 |
| FN (atom% excess) | 0.183 ± 0.282 | 0.002 – 1.305 | 0.103 | 0.133 |
| $^{15}$N tracer recovery (%) | 0.002 ± 0.004 | 0 – 0.018 | 0 | 0.002 |

***Table 8*** *Root colonisation levels found in greenhouse-grown Pinus sylvestris seedlings exposed to different ectomycorrhizal fungi treatments. Harvests were carried out at 11 and 14 weeks. Abbreviations stand for "fu0" = control seedlings, "Lb4" = inoculation with 4 ml of Laccaria bicolor, "Lb8" = inoculation with 8 ml of L. bicolor, "Sv4" = inoculation with 4 ml of Suillus variegatus, and "Sv8" = inoculation with 8 ml of S. variegatus.*

| Harvest | Treatment | n | Mean ± SD | Range | Median | IQR |
|---|---|---|---|---|---|---|
| First | fu0 | 6 | 6.0 ± 7.5 | 0 – 18.4 | 2.8 | 9.2 |
| | Lb4 | 6 | 17.3 ± 11.5 | 3.2 – 29.9 | 18.8 | 19 |
| | Lb8 | 6 | 18.5 ± 15.3 | 0 – 34.1 | 23.2 | 25.2 |
| Second | fu0 | 6 | 2.3± 5.7 | 0 – 14 | 0 | 0 |
| | Lb4 | 6 | 82.7 ± 11.5 | 67 – 97 | 81.5 | 15.5 |
| | Lb8 | 6 | 91.3 ± 8.3 | 79 – 100 | 93.5 | 11.8 |
| | Sv4 | 6 | 60.8 ± 38.4 | 0 – 94 | 69.5 | 51.3 |
| | Sv8 | 6 | 75.3 ± 7.2 | 68 – 89 | 74.5 | 3.3 |

***Table 9*** *Root colonisation, growth and N/$^{15}$N uptake measurements for ectomycorrhizal and non-ectomycorhizal greenhouse-grown Pinus sylvestris seedlings from two harvests (11 and 14 weeks).*

| | | Mycorrhizal (n = 33) | | | | Non-mycorrhizal (n = 7) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Harvest** | **Variable** | **Mean ± SD** | **Range** | **Median** | **IQR** | **Mean ± SD** | **Range** | **Median** | **IQR** |
| First | Root colonisation (%) | 21.5 ± 10.8 | 3.2 – 34.1 | 25.917 | 15.086 | 0 ± 0 | 0 – 0 | 0 | 0 |
| | Total dry weight (mg) | 36.61 ± 10.168 | 20.6 – 55.6 | 34.75 | 9.15 | 41.75 ± 2.333 | 40.1 – 43.4 | 41.75 | 1.65 |
| | Shoot dry weight (mg) | 24.9 ± 6.208 | 14 – 34 | 24.5 | 7.5 | 28 ± 2.828 | 26 – 30 | 28 | 2 |
| | Root dry weight (mg) | 11.71 ± 5.353 | 5 – 21.6 | 12.25 | 6.5 | 13.75 ± 5.162 | 10.1 – 17.4 | 13.75 | 3.65 |
| | Root mass fraction | 0.312 ± 0.089 | 0.172 – 0.434 | 0.326 | 0.125 | 0.326 ± 0.105 | 0.252 – 0.401 | 0.326 | 0.075 |
| | Shoot N concentration (%) | 0.023 ± 0.01 | 0.012 – 0.038 | 0.022 | 0.015 | 0.022 ± 0.012 | 0.013 – 0.03 | 0.022 | 0.009 |
| | Shoot N content (mg) | 0.001 ± 0 | 0 – 0.001 | 0.001 | 0 | 0.001 ± 0 | 0 – 0.001 | 0.001 | 0 |
| | FN (atom% excess) | 0.398 ± 0.404 | 0.006 – 1.294 | 0.352 | 0.336 | 0.997 ± 0.833 | 0.408 – 1.586 | 0.997 | 0.589 |
| | 15N tracer recovery (%) | 0.062 ± 0.062 | 0 – 0.192 | 0.059 | 0.064 | 0.146 ± 0.091 | 0.082 – 0.21 | 0.146 | 0.064 |
| Second | Root colonisation (%) | 80.9 ± 15.7 | 39 – 100 | 84 | 18.5 | 0 ± 0 | 0 – 0 | 0 | 0 |
| | Total dry weight (mg) | 33.017 ± 24.204 | 5.8 – 118 | 26 | 21.2 | 42.6 ± 15.307 | 21 – 58 | 45 | 21 |
| | Shoot dry weight (mg) | 21.67 ± 15.213 | 5.7 – 72 | 16 | 14.5 | 27.2 ± 12.716 | 11 – 40 | 25 | 20 |
| | Root dry weight (mg) | 11.348 ± 9.491 | 0.1 – 46 | 8.6 | 8.55 | 15.4 ± 3.847 | 10 – 20 | 15 | 4 |
| | Root mass fraction | 0.32 ± 0.108 | 0.017 – 0.467 | 0.319 | 0.115 | 0.383 ± 0.088 | 0.273 – 0.476 | 0.412 | 0.134 |
| | Shoot N concentration (%) | 0.02 ± 0.009 | 0.008 – 0.044 | 0.021 | 0.011 | 0.025 ± 0.01 | 0.011 – 0.036 | 0.028 | 0.014 |
| | Shoot N content (mg) | 0 ± 0 | 0 – 0.002 | 0 | 0 | 0.001 ± 0.001 | 0 – 0.001 | 0 | 0.001 |
| | FN (atom% excess) | 0.326 ± 0.462 | 0.008 – 1.677 | 0.085 | 0.439 | 0.582 ± 0.275 | 0.297 – 0.921 | 0.554 | 0.338 |
| | 15N tracer recovery (%) | 0.055 ± 0.105 | 0.001 – 0.356 | 0.003 | 0.073 | 0.107 ± 0.082 | 0.017 – 0.212 | 0.1 | 0.084 |

***Table 10*** *Root colonisation, growth and N/$^{15}$N uptake measurements for ectomycorrhizal greenhouse-grown Pinus sylvestris seedlings inoculated with one of two ectomycorrhizal fungi species: Laccaria bicolor or Suillus variegatus. Seedlings were 14 weeks old at the time of harvest.*

| | ***Laccaria bicolor*** **(n = 21)** | | | | ***Suillus variegatus*** **(n = 11)** | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **Mean ± SD** | **Range** | **Median** | **IQR** | **Mean ± SD** | **Range** | **Median** | **IQR** |
| Root colonisation (%) | 57.2 ± 35.0 | 3.2 – 100 | 71.5 | 62.48 | 74.3 ± 18.0 | 39 – 94 | 75 | 20.5 |
| Total dry weight (mg) | 29.05 ± 12.893 | 5.8 – 55.6 | 29.8 | 14.875 | 44.218 ± 29.548 | 17.8 – 118 | 30 | 30.5 |
| Shoot dry weight (mg) | 19.427 ± 8.355 | 5.7 – 34 | 18 | 10.75 | 29.091 ± 18.333 | 10 – 72 | 23 | 20.5 |
| Root dry weight (mg) | 9.623 ± 5.375 | 0.1 – 21.6 | 9.1 | 7.675 | 15.127 ± 11.881 | 2.8 – 46 | 14 | 9 |
| Root mass fraction | 0.31 ± 0.105 | 0.017 – 0.441 | 0.325 | 0.111 | 0.332 ± 0.095 | 0.157 – 0.467 | 0.319 | 0.117 |
| Shoot N concentration (%) | 0.019 ± 0.008 | 0.009 – 0.038 | 0.021 | 0.01 | 0.024 ± 0.011 | 0.008 – 0.044 | 0.023 | 0.012 |
| Shoot N content (mg) | 0 ± 0 | 0 – 0.001 | 0 | 0 | 0.001 ± 0.001 | 0 – 0.002 | 0.001 | 0.001 |
| FN (atom% excess) | 0.282 ± 0.445 | 0.006 – 1.677 | 0.08 | 0.355 | 0.493 ± 0.413 | 0.018 – 1.097 | 0.463 | 0.664 |
| 15N tracer recovery (%) | 0.033 ± 0.052 | 0 – 0.192 | 0.004 | 0.059 | 0.116 ± 0.139 | 0.001 – 0.356 | 0.074 | 0.105 |

***Table 11*** *Root colonisation, growth and N/$^{15}$N uptake measurements for 36 ectomycorrhizal greenhouse-grown Pinus sylvestris seedlings inoculated with Laccaria bicolor. Seedlings were 11 weeks old at the first harvest and 14 weeks old at the second harvest.*

| | First harvest (11 weeks) | | | | Second harvest (14 weeks) | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **Mean ± SD** | **Range** | **Median** | **IQR** | **Mean ± SD** | **Range** | **Median** | **IQR** |
| Root colonisation (%) | 13.9 ± 12.5 | 0 – 34.1 | 12.536 | 24.122 | 58.8 ± 42.1 | 0 – 100 | 77.5 | 89 |
| Total dry weight (mg) | 43.983 ± 26.718 | 15.1 – 128 | 37.15 | 18.275 | 29.111 ± 15.193 | 5.8 – 58 | 26.6 | 17 |
| Shoot dry weight (mg) | 28.056 ± 15.341 | 10 – 80 | 25.5 | 8.25 | 18.633 ± 10.173 | 5.7 – 40 | 16.5 | 9.5 |
| Root dry weight (mg) | 15.928 ± 21.505 | 3.1 – 99 | 11.15 | 10.65 | 10.478 ± 5.803 | 0.1 – 20 | 10.5 | 9.075 |
| Root mass fraction | 0.322 ± 0.162 | 0.045 – 0.773 | 0.32 | 0.176 | 0.337 ± 0.115 | 0.017 – 0.476 | 0.347 | 0.115 |
| Shoot N concentration (%) | 0.023 ± 0.009 | 0.012 – 0.038 | 0.023 | 0.017 | 0.019 ± 0.008 | 0.009 – 0.036 | 0.021 | 0.011 |
| Shoot N content (mg) | 0.001 ± 0 | 0 – 0.002 | 0.001 | 0 | 0 ± 0 | 0 – 0.001 | 0 | 0 |
| FN (atom% excess) | 0.579 ± 0.544 | 0.006 – 1.586 | 0.413 | 0.924 | 0.279 ± 0.443 | 0.008 – 1.677 | 0.08 | 0.262 |
| 15N tracer recovery (%) | 0.085 ± 0.081 | 0 – 0.234 | 0.071 | 0.109 | 0.034 ± 0.059 | 0.001 – 0.212 | 0.003 | 0.022 |

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