Effects of nitrogen and sawdust on mycorrhizal colonization in blueberry (*Vaccinium* spp.) roots

Hanna Thomasson

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Swedish University of Agricultural Sciences, SLU
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*Effekter av kväve och sågspån på mykorrhizakolonisering i blåbärsrötter (*Vaccinium *spp.)*

Hanna Thomasson

Supervisor:	**Siri Caspersen, Swedish University of Agricultural Sciences, Department of Biosystems and Technology**
Examiner:	Malin Hultberg, Swedish University of Agricultural Sciences, Department of Biosystems and Technology

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Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Sciences
Department of Biosystems and Technology

Abstract

Blueberry plants and fungi form root symbioses, ericoid mycorrhizas, which can enhance plant growth, increase yields and possibly facilitate the transition to organic production and reduce the use of mineral nitrogen fertilizers. The outcome of this symbiosis depends on several factors such as nitrogen fertilizer dose and soil amendments, e.g. sawdust. The present project examined root samples from three blueberry cultivars, inoculated with ericoid mycorrhizal products, given two different doses of nitrogen, and grown in a substrate with or without amendment with sawdust from spruce, for mycorrhizal root colonization. Main effects show that the lower dose of N resulted in higher levels of colonization by ericoid mycorrhiza and so did the treatment with a substrate without amendment with sawdust. The interaction also showed that the treatment with the low nitrogen dose and a substrate without amendment showed higher colonization levels in comparison with any other combination of treatments, suggesting a negative synergistic effect. Multiple reasons for these decreases in colonization levels will be discussed.

Keywords: blueberry, ericoid mycorrhiza, mycorrhizal colonization, nitrogen fertilization, sawdust amendment

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1. Introduction

1.1 Ericoid mycorrhiza

Blueberry (*Vaccinium* spp.) belongs to the family Ericaceae (SLU Artdatabanken 2026). Most species of this family grow in distinctive heathlands characterized by acidic soils with low nutrient content and rich in organic matter (Smith and Read 2008). In such soils, nitrogen (N) and phosphorus (P) are the nutrients that limit plant growth the most since they primarily occur in the form of organic compounds or macromolecules which are not directly accessible to plants (Smith and Read 2008).

A solution to this problem for ericaceous plants is to associate with fungi forming ericoid mycorrhiza (ErM), a specialized, mutually beneficial root symbiosis between plants and fungi (Smith and Read 2008). The host plants belong to the order Ericales, in the northern hemisphere to the family Ericaceae which among others include the genera *Vaccinium* and *Calluna* (Smith and Read 2008). The fungi predominantly belong to different genera of Ascomycota, for example *Hyaloscypha* or *Oidiodendron* or, in some cases, Basidiomycota, for example *Kurtia* (Vohnik 2020).

The root systems of ericaceous plants lack root hairs and instead have thin distal roots, called hair roots (Smith and Read 2008). Due to this lack of root hairs blueberry plants have reduced absorption of water and nutrients (Read 1996, Tang et al. 2025). Ericaceous plants have coevolved with ericoid mycorrhizal fungi (ErMF), solving both the problem of reduced nutrient uptake and scarcity of plant accessible nutrients in soil (Smith and Read 2008).

Ericoid mycorrhizal fungi colonize epidermal cells of hair roots (Smith and Read 2008). Colonization starts with the hyphae of ErMF spreading over the surface of hair roots, then penetrating epidermal cells and finally colonizing these cells forming dense intracellular coils (Read 1996, Smith and Read 2008). These coils enable effective transfer of nutrients between plant and fungus.

Eventually, the epidermal cells of hair roots become completely occupied by hyphal coils and as the roots age the plant cells start to degenerate followed by degeneration of the fungal cells and finally a total cell breakdown occurs (Smith and Read 2008). The roots then lose their epidermal layer, and colonization is no longer possible. The life span of a colonized epidermal cell is about five or six weeks (Smith and Read 2008).

Ericoid mycorrhizal fungi have saprotrophic capacity and produce enzymes that decompose complex organic compounds (Smith and Read 2008). The enzymes, e.g. proteases and phosphatases, are hydrolytic and oxidative and release organically bound nutrients such as nitrogen and phosphorus, to the fungi which, in turn, transfer the nutrients to the plants (Bajwa et al. 1985). Ericoid mycorrhizal fungi can assimilate nitrogen as nitrate, ammonium and amino acids (Bajwa and Read 1986).

Ericoid mycorrhizal roots are superficially distributed in the soil profile (Read 1996). The decomposition ability, of course, has the greatest benefit in the top layer of the soil where matter that contains organic compounds is most abundant. Due to their saprotrophic capacity, ErMF may increase uptake of soil N (Yang et al. 2002) and may also increase both N and P content in plant tissues (Cai et al. 2021).

The fungi, on their hand, need carbon (C) to produce energy for growth, maintenance and enzyme production (Sinsabaugh et al. 2013). They acquire carbon as carbohydrates from the photosynthesis of the plants (Smith and Read 2008) or from decomposing organic matter (Clocchiatti et al 2023). Generally, in mycorrhizal associations, the amount of carbon transported from plant to fungal partner can be substantial, ranging from 4 to 20% of the plant’s C budget (Johnson et al. 1997). Living organisms, including fungi, need nitrogen to support their enzyme production and other metabolic processes (Taiz et al. 2023).

A way to analyze mycorrhizas from a plant perspective is by using a cost:benefit model (Johnson et al. 1997). The cost being allocation of photosynthates to the fungus and the benefit nutrient acquisition. Mycorrhizas are beneficial to plants when benefits exceed costs. In natural ecosystems, plant fitness (measured in survival and reproduction) determines whether maintaining a mycorrhizal partnership is advantageous (Johnson et al. 1997).

Aside from nutrient acquisition, ericoid mycorrhiza can have other positive effects. Mycorrhizal colonization can enhance plant tolerance to abiotic and biotic stress. The ability of ErMF to decompose organic matter helps in detoxification of aromatic compounds (Smith and Read 2008). Mycorrhizal fungi can also develop resistance to toxic metals, and this resistance may help ericaceous plants colonize polluted soils (Cairney and Meharg 2003). Chen et al. (2002) investigated six ErMF isolates and concluded that all of them were considerably resistant to water stress and suggested that this is of importance to host plants under drought conditions. Inoculation with different ErMF improved drought resistance in velvetleaf blueberry (*Vaccinium myrtilloides*) (Mu et al. 2021).

Salhi et al. (2021) found that ErMF can inhibit mycelial growth of pathogenic fungi in cranberry (*Vaccinium macrocarpon* Aiton) roots and Grunewaldt-Stöcker et al. (2013) found that all tested ErMF reduced pathogenic infections in the roots of heather (*Calluna vulgaris)* and hairy alpenrose (*Rhododendron hirsutum*). The features of ErM described above are essential in giving ericaceous plants a competitive advantage in their ecological niche (Smith and Read 2008).

Studying ErM shrubs in natural habitats, Bonilla et al. (2025) found that ErM increased soil C/N ratios which led to slower decomposition. They also found that ErM increased levels of total particulate organic matter C and concluded that ErM have a large, and different from other mycorrhizas, effect on soil organic matter in creating aggregates and increasing porosity and water holding capacity. Ericoid mycorrhiza reduce microbial turnover in soil by inducing N limitation on microbes and hence enhance carbon sequestration (Bonilla et al. 2025). These effects of ErM can extend into the mineral soil and not just the organic horizon.

The poor nutrient status of the soils in combination with, in some cases, toxic metals and ericaceous plant litter that contain phenolic and aromatic substances that can inhibit decomposition make ericoid mycorrhizal symbioses important for the survival of ericaceous plants and for the exclusion of other plants (Cairney and Meharg 2003).

The advantages ErM may have can lead to benefits like enhanced plant growth and rooting, more vital plants (Yang et al. 2002, Wazny et al. 2022, Zhu et al. 2025) and an increase in yield in cultivated blueberries (Brody et al. 2019, Wazny et al. 2022).

1.2 Ericoid mycorrhiza, nitrogen fertilization and sawdust amendment

1.2.1 Recommendations on nitrogen fertilization and organic amendments

Fertilizer recommendations for cultivated blueberries with N are relatively consistent. Low to moderate doses of mineral N supplied as ammonium and adjusted to plant age, developmental stadium and time of year are commonly recommended (Caspersen et al. 2013, Smith and Jacobs 2019). Nitrogen should preferably be applied in small and frequent doses rather than fewer and larger ones (Caspersen et al. 2013).

According to Parks (2025), 40 mg N/liter substrate in container cultivation is too low and causes a decrease in plant growth. Doses above 60 mg N/liter do not cause a further increase in plant growth, while doses above 100 mg N/liter cause reduced fruit weight. Davis and Strik (2022) found, in a field study over 15 years, that nitrogen fertilization above low levels had no effect on yield and fruit quality. They investigated three different levels of nitrogen fertilizer which increased gradually from 22, 67 and 112 kg N/ha respectively in the first years to 56, 168 and 269 kg N/ha in the latter years of the study and concluded that the lowest level gave the greatest berry weight.

Organic amendment and/or mulch with organic material is also recommended (Caspersen et al. 2013, Hayden n.d.), particularly on mineral soils where there is a need to create the light, humus-rich, low-pH soils suitable for blueberry cultivation (Caspersen et al. 2014). Amending soil with sawdust may be one way to achieve this, as sawdust from conifers enhances the organic matter content and lowers soil pH (Caspersen et al. 2013, Hayden n.d). When organic material is amended to soil or used as mulch, a higher nitrogen dose is recommended to compensate for immobilization by soil microorganisms (Caspersen et al. 2013, Hayden n.d.).

1.2.2 Blueberry nitrogen uptake

Several studies on ericoid mycorrhiza in blueberry or other ericaceous plants show that organic nitrogen constitutes a major N source for mycorrhizal host plants. Substantial amounts of organically bound N can be transferred from fungus to plant (Bajwa and Read 1986). Nitrogen from organic compounds is an important source of N to mycorrhizal plants of evergreen huckleberry (*Vaccinium ovatum*) and pacific rhododendron (*Rhododendron macrophyllum*) (Rains and Bledsoe 2007). Blueberry plants (*Vaccinium corymbosum*) inoculated with ErMF acquired less ^{15}N-labeled (added as mineral fertilizer) and more soil N than did noninoculated plants (Yang et al. 2002), indicating that mycorrhizal plants can use organically bound N in soil and not just applied mineral N.

Studies on the preferred form of inorganic nitrogen for blueberry plants are inconclusive. In a hydroponic system with farkleberry (*Vaccinium arboretum*) and highbush blueberry (*Vaccinium corymbosum*), ammonium uptake was significantly greater than nitrate uptake (Poonnachit and Darnell 2004). On the other hand, in a field experiment, common bilberry (*Vaccinium myrtillus*) acquired similar amounts of N from nitrate, ammonium, amino acids and peptides both short-term and long-term (Persson et al. 2002). The same applied for a greenhouse experiment, where rabbiteye blueberry (*Vaccinium ashei*) and highbush blueberry (*Vaccinium corymbosum*) were able to acquire similar amounts of both ammonium and nitrate (Douglas et al. 2017). Inoculation with ErMF increased the capacity to utilize nitrate in cranberry (*Vaccinium macrocarpon*) (Kosola et al. 2007).

Since the typical heathlands where blueberry plants naturally grow contain nitrogen mostly in organic forms and ericaceous plants depend on mycorrhizal symbioses to get access to this nitrogen (Smith and Read 2008), fertilization with mineral nitrogen might be expected to be problematic for ErMF. It has been suggested that when fertilized with easily accessible nitrogen, e.g. ammonium or nitrate, the plant's dependence on mycorrhizal fungi decreases and the carbon cost gets too high (Smith and Read 2008). The plants are then thought to send less carbon to their fungal partners, leading to decreased colonization intensity (Smith and Read 2008).

Most studies on the effect of nitrogen fertilization on ErM colonization show a negative correlation between N dose and colonization levels. For example, ericoid mycorrhizal colonization decreased in sandy soils when total soil mineral N was high (Sadowsky et al. 2010). The effect was more distinct for N in the form of ammonium, while no significant relationship between ErM colonization and soil nitrate or inorganic N (ammonium + nitrate) was found. High doses of N fertilizers in field tend to decrease ErM colonization (Scagel and Yang 2005). A weak but significant negative relationship between the ammonium concentration in the soil and colonization levels of ErM in cranberry plants was found by Stackpoole et al. (2011). High levels of N in the pot substrate decreased ErM colonization (Jiang et al. 2024).

In a meta-analysis study of 82 different studies on arbuscular- and ectomycorrhiza in field settings, nitrogen fertilizer decreased colonization levels by an average of 15% (Treseder 2004). Higher rates of N applications resulted in lesser colonization. The variation among the included studies was, however, significant and depended mostly on experimental designs.

A few field studies, however, found that nitrogen fertilizers do not affect mycorrhizal colonization levels in roots of ericaceous plants. Johansson (1999) found no significant difference in root colonization between root samples of heather (*Calluna vulgaris*) given four different treatments of ammonium nitrate (0, 35, 50 and 70 kg/ha and year). Nor did Jeliazkova and Percival (2003) in wild blueberry (*V. angustifoilium*) given 0 or 35 kg N/ha of nitrogen in the form of urea. They did however find a slight increase in mycorrhizal colonization with time in the plants given the high nitrogen dose.

Also in natural habitats, nitrogen fertilization can increase mycorrhizal colonization. At Whim Bog (an experimental site where long-term N deposition is monitored), ErMF colonization of roots of heather (*Calluna vulgaris*) and cross-leaved heath (*Erica tatralix*) increased under different forms of nitrogen application for a period of 14 years (Kiheri et al. 2020).

Colonization levels were also found to be higher under organic fertilizers than under inorganic ones but varied considerably between different cultivars and fungi (Scagel 2005). She suggested that high levels of available nutrients may negatively influence colonization and that plants grown with organic fertilizer might have been nutrient limited and therefore were more intensely colonized.

Too low levels of soil N may also decrease mycorrhizal colonization levels. In a field experiment, Yang et al. (2002) found that insufficient levels of N in the soil decreased ErM formation and suggested that this was due to reduced plant growth and root length, caused by N stress.

1.2.3 Sawdust amendment

Ericaceous plants and their symbiotic fungal partners thrive in soils with a high content of organic matter. In a formosan rhododendron (*Rhododendron formusanum*) forest, the organic matter C content was around 50%, the content of phenolic substances around 30% and the C/N ratio close to 50 (Lin et al. 2011). These circumstances indicate that ericoid mycorrhizal fungi are well adjusted to such conditions. A way to enhance the organic matter content in soil or substrate in blueberry cultivation is soil- or substrate amendment with sawdust. Sawdust shares many of the described characteristics (Goh and Haynes 1977).

A few studies on how sawdust amendment affect mycorrhizal colonization have been conducted. Plants grown in soil amended with rotted sawdust had lower growth than plants in soil with amendment of forest litter or no amendment (Yang et al. 2002). Inoculated plants treated with rotted sawdust also had the lowest mycorrhizal colonization levels.

Tang et al. (2025) tested the northern blueberry highbush cultivar ‘Bluecrop’ (*Vaccinium corymbosum*) and the half-high cultivar ‘Northland’ (*V. corymbosum*) both in pots and in field. They found that the colonization levels for both cultivars were higher in the field trial when sawdust was mixed into the soil than for the unamended black loam control. No significant difference was found between the control and the soil amended with peat and sawdust. In the pot trial, however, both amendment with sawdust and sawdust combined with peat generated lower colonization levels than the unamended black loam control.

1.3 Environmental impact

The United Nations has formulated seventeen sustainability goals aimed “to secure the rights and well-being for everyone on a healthy, thriving planet’’ (United Nations n.d.). Several of the goals (e.g. goal 2: zero hunger, goal 12: responsible consumption and production, goal 13: climate action and goal 15: life on land) pertain to sustainable food production, which includes organic production (United Nations n.d.).

Nitrogen fertilization has considerable effects on climate and environment and accounts for 2.1% of the world’s total greenhouse gas emissions (Menegat et al. 2022). The emissions of greenhouse gases originate from the production of fertilizers that release N_2O into the atmosphere and production also requires a lot of energy, often as fossil fuels that release CO_2 (Menegat et al. 2022). Transportation and distribution also require energy which release more CO_2, and application in field leads to emissions of more N_2O (Menegat et al. 2022). Moreover, emissions of NH_3 into the atmosphere and leaching of NO_3^- to the groundwater have major environmental impact and are a result of application in field (Tyagi et al. 2022).

Fertilizer application (and fossil fuel burning) have enhanced the levels of reactive nitrogen in circulation (Erisman et al. 2011), altering the nitrogen cycle in ways harmful to climate, human health, food security and ecosystem services. Anthropogenic nitrogen also alters the nutrient status and nitrogen sources of ericaceous plants in peatlands and may accelerate peat carbon loss (Vesala et al. 2021).

Since ericoid mycorrhizal fungi enhance plant uptake of organically bound nutrients (Smith and Read 2008), increase plant stress tolerance (Chen et al. 2002, Salhi et al. 2021) and, in combination with endophytic fungi, can be an alternative to conventional fertilization and pesticide application, ErM may contribute to the conversion of conventional blueberry production towards organic production (Wazny et al. 2022). Ericoid mycorrhizal symbioses may be a way of reducing the use of nitrogen fertilizer and shift the production of blueberries (and other ericaceous plants) towards organic production.

However, many factors, e.g. soil properties and fertilizer, cultivar and fungal species, influence the effect of ErM (Caspersen et al. 2016). A need for research that maps interactions between these factors and methods for combining cultivars and fungi under different conditions is evident.

1.4 Objectives

The purpose of the present project was to evaluate the effects of nitrogen dose and amendment with sawdust on mycorrhizal colonization levels of roots of blueberry (*Vaccinium* spp.). The roots came from a study on the effects of blueberry cultivar, mycorrhizal inoculum, nitrogen fertilizer dose and sawdust amendment on the establishment of blueberry plants and mycorrhizal colonization levels that was conducted in 2011 (Caspersen et al. 2014). Root samples were collected and stored.

The questions posed for this project were:

- How do different doses of nitrogen fertilizer impact colonization levels of ErMF?
- How does amendment with sawdust from spruce impact colonization levels of ErMF?
- Are there any interaction effects between nitrogen and sawdust?

The present project did not include effects of cultivar or mycorrhizal treatment on mycorrhizal colonization.

2. Materials and methods

2.1 Plant and fungal material

The plant material used in this study was root samples collected from three different blueberry cultivars, *Vaccinium corymbosum* cv. Duke', *V. corymbosum* cv. 'Reka' and *V. angustifolium x V. corymbosum* cv. 'North Blue'. Micropropagated plants were transferred to seed trays with a peat substrate and given one of three different mycorrhizal treatments: control, Rhodovit (Symbiom) and Rhodazo (INOQ). In addition, the plants were given two different doses of nitrogen, 35 or 105 mg per liter of substrate. After about six weeks the plants were transferred to pots with the same substrate and fertilizer treatment, but half of the pots were amended with 10% sawdust from spruce.

The plants were kept in a greenhouse at Elitplantstationen in Kristianstad, Sweden. The root samples used in the present project were collected in September of 2011 (the first growing season). They were cleaned and stored in ethanol (50%) in plastic jars until January of 2026 when this project started. Each treatment contained three replicates and their sizes were equal. In total, 108 root samples were collected.

2.2 Preparation of root samples and assessment of root colonization

To investigate whether the roots and fungi had formed ericoid mycorrhiza, an assessment of root colonization was made. A representative subsample from each root sample was made using the quartering-method. Each sample was carefully spread and divided into sixteenths and from each of the sixteenths a small subsample was collected and put in a plastic jar.

The method used for clearing and staining the root subsamples was modified from Vohnik (2020) and Waterman (2015). To clear the subsamples from natural pigments they were immersed in 10% KOH, heated in a microwave oven until just below the boiling point and left for 24 hours and then rinsed thoroughly with deionized water until the rinse water was clear. To clear any remaining pigments, the roots were soaked in room temperature with 12% H_2O_2 for 10 minutes. Then the roots were rinsed thoroughly again before being immersed in 1% HCl at room temperature overnight in order to acidify them.

To visualize the mycorrhizal fungi, a solution of ink, glycerol, 1% HCl and deionized water was used. The root subsamples were immersed in the ink solution and left in room temperature for one hour. The ink solution was then poured out and replaced with a solution without ink, containing glycerol, HCl and deionized water for 2-3 days.

The individual roots were then carefully spread on a microscope slide with a few drops of the solution, and a cover slip was mounted. To assess root colonization, the presence of intracellular hyphal coils (figure 1, B and C) was examined using the magnified intersections method, which involved examining vertical transects that intersected with root segments along each slide. Each intersection was scored with or without hyphal coils. At least 200 intersections per sample were scored.

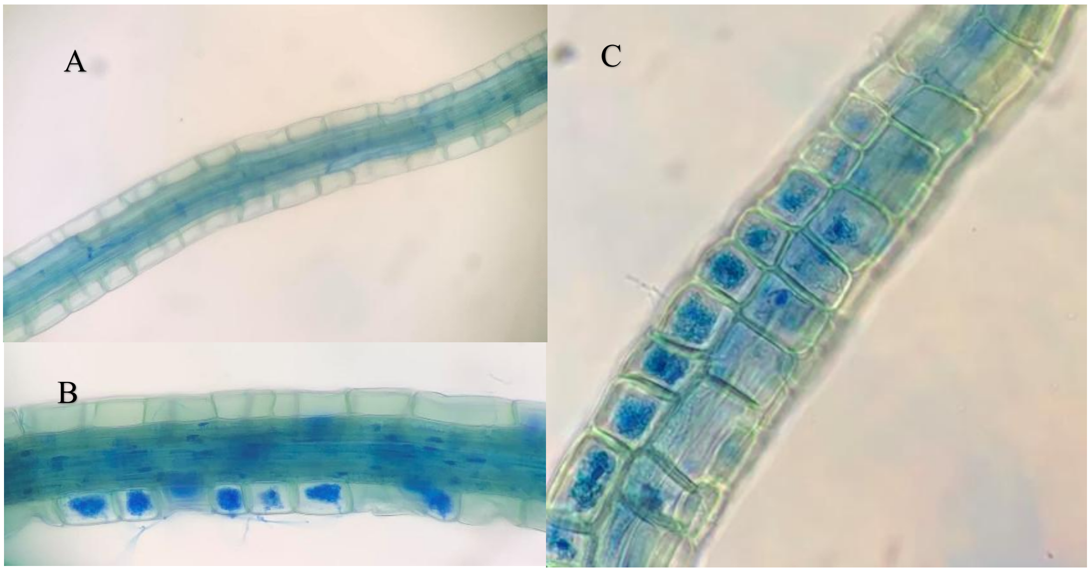

Figure 1. Hair roots of blueberry plants. A: An uncolonized root. B and C: Colonized roots with intracellular hyphal coils. [photographs].

2.3 Statistical analysis

Data from the assessment of root colonization was compared based on nitrogen dose and sawdust amendment using two-way ANOVA (analysis of variance) in Minitab 21. ANOVA assumptions were assessed, by Grubbs' test for significant outliers, Anderson-Darlings' test for normality, and Levens' test for homogeneity of variances. Values with large residuals were double checked. Moreover, significant differences were identified using Tukey's pairwise comparisons. All analyses were run at a significance level of 5%. Graphs and figures were created using Excel (Microsoft 365, version 2604).

3. Results

All examined root samples were colonized by mycorrhizal fungi. Colonization levels were significantly higher ($p<0.05$) in roots of blueberry plants treated with the low dose of nitrogen (35 mg N/liter substrate) than those treated with the high dose (105 mg N/liter substrate) (figure 2). The low dose of nitrogen rendered around 16% higher colonization levels than did the high dose when treatment means were compared.

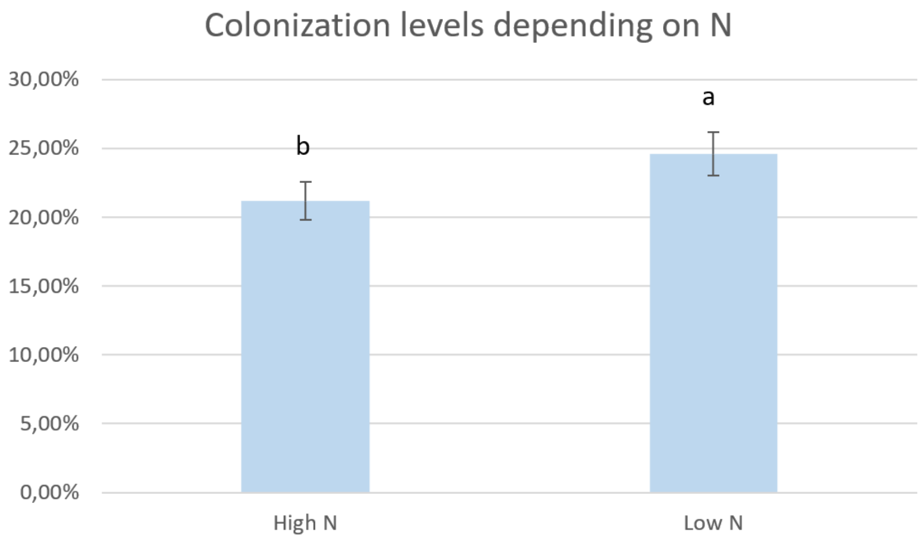

Figure 2. Colonization levels in the roots of blueberry plants treated with either 105 or 35 mg N/liter substrate. Means ± se of 54 samples.

The effect on colonization levels of sawdust amendment was also significant ($p<0.01$) (figure 3). No amendment with sawdust rendered around 33% higher colonization levels than did amendment with 10% sawdust from spruce when treatment means were compared.

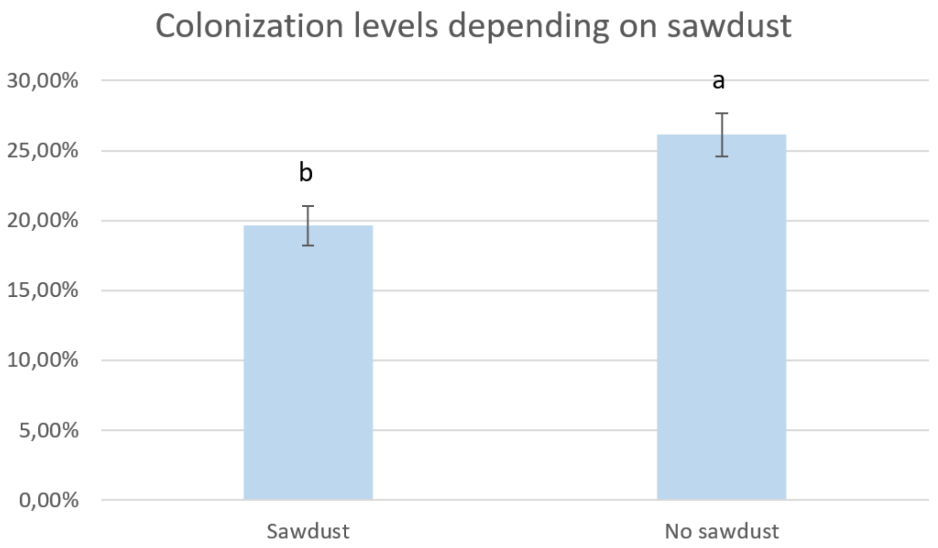

Figure 3. Colonization levels in the roots of blueberry plants treated with either 10% sawdust from spruce or no amendment. Means ± se of 54 samples.

Interaction effects between nitrogen dose and sawdust amendment were also significant. Colonization levels were significantly higher (p<0.05) in roots of blueberry plants treated with the low dose of nitrogen and grown in a substrate without amendment than any of the other treatments (figure 4). Colonization levels were between 29% (compared to high N and no sawdust) and 51% (compared to high N and sawdust) higher when treatment means were compared. The three other treatments did not significantly differ from one another.

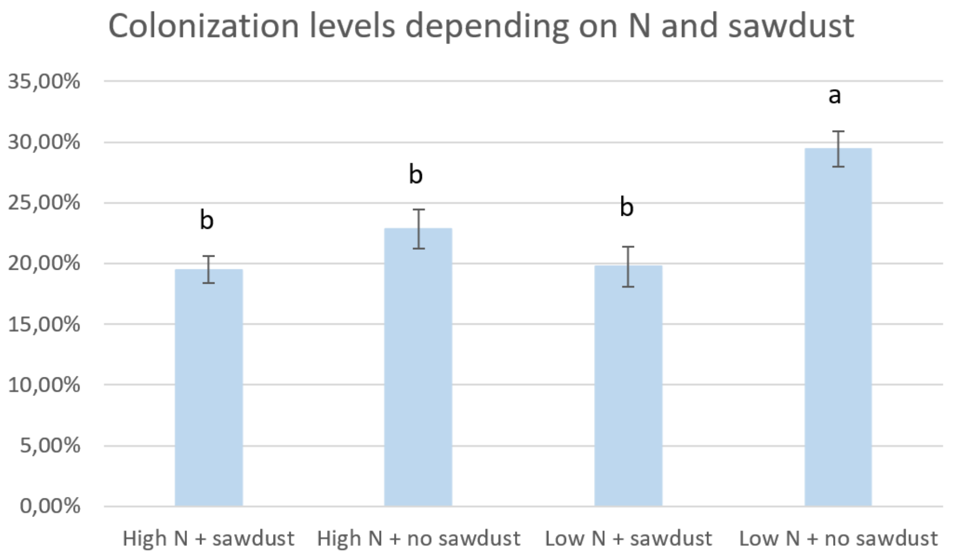

Figure 4. Colonization levels in the roots of blueberry treated with different doses of nitrogen and sawdust amendment. Means ± se of 27 samples.

4. Discussion

The purpose of this study was to evaluate the effects of nitrogen fertilizer dose and sawdust amendment on mycorrhizal colonization levels in blueberry roots. The differences in colonization levels for the investigated treatments were significant and substantial. The differences in colonization levels of 16% to 51% found in this study (figure 2, 3 and 4) might be of practical relevance in blueberry production. For example, higher ErM colonization resulted in greater enzyme production which led to measurably higher uptake of organic nitrogen and increased biomass production in ericaceous plants (Cairney and Meharg 2003, Grelet et al. 2009). Higher N uptake also led to increased fruit yield and berry numbers (Fang et al. 2020).

Similar effects on ErM colonization have been shown in several studies for nitrogen alone (Sadowsky et al. 2010, Scagel and Yang 2005, Jiang et al. 2024) and for sawdust alone (Yang et al. 2002, Tang et al. 2025). The colonization levels of ericoid mycorrhiza, however, depend on many other factors than nitrogen and sawdust and these factors may also affect the interaction between N and sawdust. Furthermore, the colonization responses to nitrogen and/or sawdust vary between pot- or field experiments (Tang et al. 2025) and over time (Yang et al. 1998). Cultivar and fungal species (Neves et al. 2025), soil conditions such as pH (Leake and Read 1990) or soil moisture (Kohout and Tedersoo 2017), and other growing conditions such as light (Johnson et al. 1997) may also influence colonization levels of ericoid mycorrhiza.

A few reasons for the varying results of previous research are possible. Experiments in fields and natural habitats are likely more difficult to control since many factors interact and influence the effect of nitrogen fertilization and sawdust amendment, and these factors probably vary substantially between different fields and habitats in different experiments. In fields and natural habitats there is, for example, probably more nutrient competition between plants, fungi, other plants (e.g. weeds) and microorganisms. Experimental design varies greatly in pot and greenhouse experiments (Treseder 2004) and might cause differences in mycorrhizal responses.

4.1 Effects of nitrogen

According to Parks (2025), around 50-55 mg N/liter is an optimal dose for plant growth during the establishment phase. The blueberry plants in this study were given an N dose that was either below or above the optimum for plant growth, but they were not given an optimal dose. The colonization levels that an optimal N dose for growth would lead to remain unknown.

Some studies have shown that plant growth does correlate with the presence of mycorrhizal colonization, but not with the intensity of colonization (Neves et al. 2025, Carrillo et al. 2015), meaning that colonized plants grow better but that higher colonization levels do not necessarily cause higher growth. Other studies have, however, shown that variation in colonization levels causes variation in N uptake and that higher colonization levels lead to enhanced shoot- and root biomass (Cairney and Meharg 2003, Grelet et al. 2009). It would be interesting to further evaluate whether the colonization levels in this study impacted the plant growth and whether mycorrhizal colonization could compensate for the low N dose.

Several reasons behind the observed decline in colonization under high mineral N supply (figure 2) might be plausible. Although they work through different mechanisms, most of them probably lead to the suggested outcome: when mineral nitrogen is abundant, the plant's dependence on its fungal partner decreases (Smith and Read 2008). As a result, carbon allocation to the fungus is reduced and colonization levels consequently fall (Smith and Read 2008). Mycorrhiza is a costly symbiosis where the plant must actively export carbohydrates to the fungus to keep the symbiosis going (Johnson et al. 1997).

One plausible reason for the decrease in colonization levels is that mineral nitrogen suppresses the production and activity of some fungal extracellular enzymes used to break down complex nitrogen compounds (Cairney and Meharg 2003). Less fungal enzymes will probably lead to less decomposition of organic compounds and less nutrient transport to plants, and the cost of the symbiosis outweighs the benefit and colonization declines.

In a liquid culture system with a basal medium without nitrogen, the colonization levels in roots of the ericaceous plant salal (*Gaultheria shallon*) were lower when ammonium or glutamine was added to the medium and higher when peptide, protein, or no nitrogen was added (Xiao and Berch 1999). When the medium contained protein and peptides, the enzymes of the fungi were probably crucial in making the N accessible to plants and led to fungal growth and higher colonization levels, while the opposite was true when the medium contained ammonium or the amino acid, since those are already accessible to plants. Plants, not inoculated with ErMF were unable to use complex nitrogen sources (Xiao and Berch 1999).

Another possible reason could be that high levels of mineral N alter rhizosphere conditions, including pH, which can further influence mycorrhizal colonization.

Soil pH decreased with increased N levels when N was applied as NH_4NO_3 (Jiang et al. 2024). Fertilizing with ammonium alone has a strong acidifying effect while nitrate has a more moderate alkaline effect (Eriksson et al. 2011).

Ericoid mycorrhizal fungi grow best at a pH value of 4-5 (Bajwa et al. 1985). A lower pH led to a decrease in the activity of N-related enzymes (Jiang et al. 2024) and negatively affected the fungal production of enzymes that break down protein and the activity of those enzymes (Leake and Read 1990). A pH between 1.5 and 2 completely inhibited the activity of some enzymes of ErMF (Leake and Read 1990). Without sufficient production or activity, ErMF may be unable to mobilize the organic nitrogen required to sustain carbon exchange with the host. At higher pH values (≥5), bacterial and saprotrophic communities are favored over ErMF (Leake and Read 1990), which likely reduces colonization as ErMF become competitively displaced.

Aside from the pH effect, high mineral N directly favors bacteria and saprotrophs, e.g. yeasts and moulds, over ErMF. In an N poor environment, decomposition was slower when mycorrhizal roots were present in soil than when they had been removed (Gadgil and Gadgil 1971). The authors suggested that this was due to mycorrhizal fungi outcompeting saprotrophic fungi for nitrogen. This effect is now named the Gadgil effect and was confirmed for early stages of decomposition by Stekenburg et al. (2018). This suggests that when circumstances are opposite, with abundant nitrogen, the saprotrophs will most likely outcompete mycorrhizal fungi. Under conditions of high N deposition, bacterial communities typically dominate over fungal ones (Wang and Kuzyakov 2024). Nitrogen in the form of ammonium or nitrate stimulated bacteria and fast-growing fungi which outcompeted the slower growing ErMF for carbon, space and root contact (Polussa et al. 2024).

Nitrogen fertilization can also change the species composition of mycorrhizal communities (Johnson et al. 1997), which might lead to suppression of colonization depending on fungal species. This might be due to differences in nitrogen use between fungal species. Ericoid mycorrhizal fungi differed in their utilization of various N sources under low C availability, and this difference was maintained in symbioses with host plants (Grelet et al. 2009).

Another possible explanation for the decrease in colonization that is not related to the previously described mechanism might be that an increase in root biomass under nitrogen fertilization may contribute to lower percentage colonization through a dilution effect. Generally, root biomass increases slightly under N fertilization (Li et al. 2015, Wang and Iiu 2014). High soil N levels increased root tissue density and root average diameter but decreased root length in *Vaccinium* *corymbosum* (Jiang et al. 2024). Most studies on effects of N fertilizer on mycorrhizal colonization do not report any changes in root architecture but focus on other explanations (Scagel and Yang 2005, Vesala et al. 2021). The contribution of larger root systems is therefore probably small but may not be ruled out.

Other nitrogen sources than fertilization might also influence ericoid mycorrhizal colonization. Hydrologic inputs and organic matter from stem and leaf litter contributed substantially, about 15%, to the N levels in soil in a field experiment on cranberry (*Vaccinium sp*.) (Stackpoole et al. 2011). They suggest that growers should consider all nitrogen sources, not just fertilizers, to avoid excess fertilization and to keep optimal levels of ErM.

The conclusion that increasing soil N levels lead to a decrease in mycorrhizal root colonization is supported by studies in natural ecosystems where plants and fungi have coevolved under nutrient-poor conditions. A long-term field experiment in heathlands (Vesala et al. 2021) as well as a meta-study across mycorrhizal types (Treseder 2004), consistently show that raised nitrogen availability suppresses mycorrhizal colonization. In commercial cultivation fields though, colonization levels depend on numerous other factors and will need further attention.

4.2 Effects of sawdust

Sawdust amendment is a way to increase the organic matter content in soil or substrate and is a common practice in blueberry production in the USA (Caspersen et al. 2013). Reasons for this recommendation is probably that sawdust incorporation alters the properties of the soil or the substrate. Sawdust amendment enhanced the total airspace and decreased the easily available water content (Goh and Haynes 1977). Furthermore, the sawdust had a pH value of 5.33, high carbon content and an extremely high C/N ratio, 6138:1. Sawdust mixed with peat had C/N-values of around 70:1 (Goh and Haynes 1977). pH will drop over time when sawdust is added to the planting hole (Ochiman et al. 2019), due to the formation of organic acids during sawdust decomposition (Liu et al. 2023). Soil carbon content increases when sawdust is amended to soil (Reichel et al. 2018).

Many of these properties are likely favorable to blueberry and ErM. Fungal decomposers are generally more effective than bacteria in the uptake of complex organic compounds and tend to be favored under acidic and drought-prone conditions (Wang and Kuzyakov 2024). The biomass and activity of arbuscular mycorrhizal fungi increased when sawdust was amended to the soil (Clocchiatti et al. 2021), which indicates that this might be true even for ErMF since they evolved under conditions like those under sawdust amendment (Smith and Read 2008).

Differences in C/N-ratio between different amendments can explain differences in plant growth and mycorrhizal colonization levels (Yang et al. 2002). The high C/N-ratio of sawdust causes immobilization of mineral nitrogen, both naturally present and added, by soil microorganisms decomposing the sawdust (Goh and Haynes 1977). The immobilization is rapid and fungal driven, and the high C/N ratio also suppresses bacterial activity (Clocchiatti et al. 2023). This leads to a decrease in plant accessible N in soil (Mooshammer et al. 2014) and could possibly benefit ErM since low N availability, as previously stated, usually does so.

Sawdust may also contain substances that favor ErMF. Ericoid mycorrhizal fungi have, as stated above, saprotrophic capabilities and can degrade lignin which can be an extra source of carbon (Bending and Read 1997). One strain of *Oidiodendron maius* grew better when the growing medium contained the flavonoids rutin and quercetin (Mikheev et al. 2023). This strain also possessed extracellular phenol oxidase activities, suggesting a capacity to decompose phenolic compounds. This ability is, however, small compared to wood decomposing fungi (Bending and Read 1997). Amendments with high C/N ratios, in combination with suitable ericaceous host plants, therefore probably favor ErMF and their colonization of ericoid roots. Ericoid mycorrhizal fungi need carbon allocation from plants to thrive and gain enough energy to grow and build mycelia (Cairney and Meharg 2003).

In contrast to the factors discussed above, this study (figure 3) and earlier ones (Yang et al. 2002, Tang et al. 2025) reported an initial decrease in mycorrhizal colonization after sawdust amendment. Several reasons for the observed decline are possible. Firstly, sawdust has very high C/N ratios which leads to extreme N immobilization. Yang et al. (2002) suggested that the reduced colonization levels were due to plant N stress caused by sawdust decomposition. If available N is so low that it reduces plant growth it may affect colonization levels negatively (Yang et al. 2002). Mycorrhizal effect on decomposition decreases with increasing C/N ratios in field experiments (Choreno-Parra and Treseder 2024). This may be a sign of decreased colonization.

Secondly, low pH inhibits productivity and activity of certain enzymes, as stated above (Leake and Read 1990, Jiang et al. 2024). Peat has a pH value of around 4-4,5 (Goh and Haynes 1977). When the peat substrate in this study, already with a low pH value, was amended with sawdust, it is possible that the pH value in the substrate decreased below critical values, due to the formation of organic acids (Liu et al. 2023) and affected plants and mycorrhizal fungi negatively.

Low pH could also lead to an increase in toxic cations, e.g. Al^{3+}, in the soil solution, leading to plant Al uptake and toxicity symptoms such as reduced growth (Alotaibi et al. 2021). They found that arbuscular mycorrhizal fungi can reduce Al accumulation in plant tissues. This mechanism is probably relevant to ErMF as well since ErM plants grow in acidic, Al rich environments. If cation levels increase above critical levels, the toxic effect on plants is likely to increase, and stressed plants are likely less supportive of mycorrhizal symbioses.

Yet another reason could be soil moisture. Most of root associated fungal communities showed affinity to sites with higher soil moisture in a field study in South Africa (Kohout and Tedersoo 2017). Only two taxa preferred drier sites. Since sawdust enhanced total airspace and reduced the water holding capacity (Goh and Haynes 1977) it is possible that the sawdust amended substrate in this study was drier and therefore less favorable to ErMF.

Further possible reasons could be that sawdust may contain substances that inhibit fungal colonization and growth. Wood of silver fir contains lignans, phenolic acids and flavonoids (Vek et al. 2021). The authors found that extracts from silver fir inhibited growth of wood decaying fungi and molds. It is possible that some of those substances also inhibit ErMF.

Sawdust may also alter the abundance and composition of fungal communities in soil. Pine sawdust amendment in former agricultural soils left fallow for 3-6 years decreased the total fungal populations after two years (Kwasna et al. 2000). Some fungal species, e.g *Trichoderma harzianum,* increased significantly while most fungal populations decreased. Ericoid mycorrhizal fungi are relatively slow growing (Vohnik et al. 2020, Mikheev et al. 2023) and may be outcompeted by faster growing saprotrophs for the carbon of the sawdust.

Some studies indicate, however, that the reduction in colonization levels may diminish over time (Yang et al. 1998). Both plant growth (Yang et al. 1998; 2002) and mycorrhizal colonization levels (Yang et al. 2002) were negatively affected by sawdust amendment during the first growing season. In the second season, however, no measurable effects on plant growth were maintained (Yang et al. 1998). Similar growth between plants in amended and unamended soil may indicate similar colonization levels, since colonization intensity positively correlate with enhanced shoot- and root biomass (Grelet et al. 2009). However, as previously mentioned, mycorrhizal colonization intensity does not always correlate with plant growth (Neves et al. 2025, Carrillo et al. 2015).

During organic matter decomposition, nutrients will eventually be mineralized and made available to plants (Ochmian et al. 2019). It is possible that the effects of sawdust amendment on colonization levels decrease over time. When the sawdust is partially decomposed, immobilization and microbial competition decrease. An N poor and C rich environment with pH levels within a suitable range, that favors ericoid mycorrhiza may then develop. Decomposition of organic matter such as sawdust may extend beyond a single growing season (Yang et al. 2002), meaning that both the chemical environment and the competitive dynamics in the rhizosphere continue to change over time.

The root samples in the present study were collected at the end of the first growing season, a point at which sawdust decomposition was likely far from complete. Roots sampled in subsequent seasons might therefore show different patterns of mycorrhizal colonization.

Davis and Strik (2022) found, in a field study over 15 years, that soil amended with sawdust had higher content of organic material and gave increased yields by 4%. Increased yields may indicate increased mycorrhizal colonization, since ErM colonization increases N uptake in plants (Cairney and Meharg 2003, Grelet et al. 2009) and higher N levels might increase yields (Fang et al. 2020).

4.3 Interactive effects between N and sawdust

The interaction between nitrogen and sawdust seems to be negatively synergistic since the treatment with low nitrogen and no sawdust amendment had the highest colonization levels and the treatment with high nitrogen and sawdust amendment had among the lowest, although not significantly so (figure 4). The effects of both nitrogen and sawdust together seemed to be stronger than the effects of each of them alone.

The interactions possibly happen through several interacting mechanisms. Sawdust raises the C/N ratio (Goh and Haynes 1977) and increases microbial competition for N (Wang and Kuzyakov 2024, Polussa et al. 2024) which enhances the plant's dependance of a functional ErM symbiosis. At the same time high N doses decrease carbon allocation to the fungus (Smith and Read 2008), which in turn limits fungal growth and enzyme production (Cairney and Meharg 2003). These processes seem to counteract, sawdust increases the plant's need for mycorrhizal function, whereas mineral N reduces the fungus' ability to sustain it.

Sawdust also stimulates saprotrophs that compete with ErMF for organic nitrogen (Mikheev et al. 2023), and high N doses favor bacteria which quickly immobilize available N (Polussa et al. 2024). These processes might amplify each other and reduce the functional space for ErMF. High mineral N also reduces fungal enzyme production (Cairney and Meharg 2003). Sawdust, on the other hand requires high enzyme activity to release organically bound nitrogen (Bending and Read 1997, Mikheev et al. 2023). The combination may therefore result in the fungus being unable to functionally respond to the increased C/N ratio. The fungus possibly becomes deprived of both carbon from the plant and nitrogen from the soil and has neither the energy nor the nutrients to grow and colonize roots.

Ericoid mycorrhiza is most favored in soils with low N, high C and high content of organic matter (Smith and Read 2008), which is the type of environment created when low nitrogen doses are applied, sawdust decomposition has been going on for some time, and the high immobilization rate slows down.

Mycorrhizal associations are very complex, and their functioning depends on several biotic and abiotic factors at the rhizosphere-, community-, and ecosystem-scales (Johnson et al. 1997). They concluded that the natural symbiosis is probably truly mutualistic, but in managed agricultural systems, where soil nutrient shortages are eliminated and plant genotypes are selected by humans and not by evolution, the symbiosis will probably become more parasitic. Today, when the need to reduce environmental impact and climate change is urgent, practices that help push the symbioses back toward its natural mutualistic function can be critically important.

4.4 Method

The method used to prepare the samples of blueberry roots is the standard procedure (Vohnik 2020) which strengthens the validity of the results. The method for clearing and staining the subsamples made it relatively easy to identify the intracellular coils of ericoid mycorrhiza (figure 1). The method does however have some limitations. Due to great variation in colonization in a single root system (Vohnik 2020) retrieving representative subsamples from the original root samples is difficult. In addition, the individual roots plated on microscope slides constitute a very small part of the subsample. Despite these difficulties, the results were relatively even and clear. This is supported by the small standard errors which indicate low within-treatment variation and the large number of replicates which indicate certainty in the means. Together, these factors suggest that the observed differences reflect true treatment effects and not random variations and strengthen the reliability of the results.

Another limitation is the difficulty in correctly identifying hyphal coils of ErM. Roots of ericaceous plants may be inhabited by other fungi, not considered true ErMF, making similar structures or hyphal coils/loops. Not every intracellular hyphal coil in the hair root of an ericaceous plant necessarily represents ErM (Vohnik 2020). This difficulty was overcome by comparative material and pictures retrieved from several other studies (Vohnik 2020, Neves et al 2025, Bizabani et al. 2016), which further strengthens the validity of the results.

No significant outliers were found with Grubbs' test. ANOVA, however, identified five values with large residuals, $|R| > 2$. These values were checked and found correct. They probably represent extremes and may be due to the difficulty of making a representable subsample from the root samples.

4.5 Implications

Although this study provides results on how nitrogen and sawdust may affect ericoid mycorrhizal colonization, several limitations restrict the strength of the conclusions that can be drawn. Only colonization was measured and not for example enzyme activity, nitrogen acquisition or carbon exchange, which means only the structure of colonization and not its function was assessed. The study also did not assess microbial community composition or pH dynamics even though both might be important mechanisms behind sawdust driven colonization reduction. This made it difficult to identify which processes actually caused the observed decline. Furthermore, the study lacked a time dimension, leaving open the question of whether ErM might recover once sawdust decomposition progresses. Finally, the study did not evaluate the effect of cultivar and fungal inoculum on colonization levels.

Many studies on the correlation between mycorrhizal colonization levels and different factors affecting them have been conducted (for example Sadowsky et al. 2010, Tang et al. 2025, Scagel and Yang 2005). A clear picture of their interactions is, however, still hard to define. Future research should therefore incorporate time-series designs, analyses of fungal and bacterial communities, measurements of enzyme activities and tracing of nitrogen and carbon flows. Experiments that investigate the effects of C/N ratios, pH, microbial competition, cultivar and fungal species would also help disentangle the different mechanisms. Meta-analysis studies might also help in clearing these intricate interactions.

This study, along with previous and further research, is relevant to several of the sustainability goals. Ericoid mycorrhiza can play a key role in nutrient efficiency (Bajwa and Read 1986, Rains and Bledsoe 2007, Yang et al. 2002) and stress tolerance (Cairney and Meharg 2003, Chen et al 2002, Salhi et al. 2021), and understanding how management practices influence this symbiosis may help in reducing fertilizer dependence, minimizing nitrogen losses and maintaining resilient ecosystems. From a practical perspective, the results suggest that combining high nitrogen doses with fresh sawdust is likely to be harmful to ericoid mycorrhiza and also to production systems that take advantage of ErM. At the same time, previous research indicates that organic amendments may enhance ErM in later stages of decomposition (Yang et al. 2002, Ochmian et al. 2019). Ericoid mycorrhizal shrubs reduce the rate of decomposition and enhance carbon sequestration (Bonilla et al. 2025, Gadgil and Gadgil 1971) which is environmentally beneficial. It is important to develop management strategies that align with ecological processes rather than counteract them.

4.6 Conclusions

High doses of nitrogen fertilization reduced ericoid mycorrhizal colonization levels in this study. Reasons for this decrease might be that the plant's need for fungal nitrogen supply declined, making the carbon cost of the symbiosis too high. The plant then decreases carbon allocation to the fungus, fungal enzyme activity is suppressed, and the rhizosphere changes in ways that reduce colonization.

Sawdust amendment also decreased ericoid mycorrhizal colonization levels in this study. This is potentially due to nitrogen immobilization and microbial competition but, as the sawdust decomposes over time, an N poor, C rich and stable rhizosphere environment that may favor ericoid mycorrhiza might develop.

The interaction effects between nitrogen and sawdust are likely negatively synergistic and high N and sawdust strongly decreased colonization levels in this study. This may, however, change over time as sawdust decomposes and an environment more suitable for ericoid mycorrhizal colonization develops.

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