Saprotrophic-ectomycorrhizal fungal interactions affect poplar performance

Article

Pena, R. ORCID: https://orcid.org/0000-0002-7985-6906, Milner, G. and Tibbett, M. ORCID: https://orcid.org/0000-0003-0143-2190 (2025) Saprotrophic-ectomycorrhizal fungal interactions affect poplar performance. Environmental and Experimental Botany, 238. 106246. ISSN 0098-8472 doi: 10.1016/j.envexpbot.2025.106246 Available at https://centaur.reading.ac.uk/124606/

To link to this article DOI: http://dx.doi.org/10.1016/j.envexpbot.2025.106246

Publisher: Elsevier

Environmental and Experimental Botany 238 (2025) 106246

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Research paper

Saprotrophic-ectomycorrhizal fungal interactions affect poplar performance

Rodica Pena [a,b,*], Gemma Milner [a], Mark Tibbett [a]

[a] Sustainable Land Management, School of Agriculture, Policy and Development, University of Reading, Reading, UK
[b] Department of Silviculture, Transilvania University of Brașov, Brașov, Romania

ARTICLE INFO

Keywords:
Decomposition
Nitrogen limitation
Nutrients
Photosynthesis
Root traits
Stable isotopes
Symbiosis

ABSTRACT

Mycorrhizal and saprotrophic fungi are key players in plant nutrition in forest ecosystems, affecting nutrient availability and plant nutrient acquisition, but the impact of their interaction on plant performance remains largely understudied. Their interaction is particularly important under nutrient-limited conditions (e.g., nitrogen limitation) as they may compete for resources or engage in facilitative interactions that ultimately affect plant nutrient uptake and growth. Here, we used a simplified, plant-centric experimental design to investigate the effects of fungal interactions on plant performance. Poplar (*Populus* × *canescens*) plantlets were grown under nutrient-poor conditions for 23 weeks with a single nutrient source: a mixture of ^{15}N-labelled poplar (labile) and beech (recalcitrant) leaf litter. Plants were inoculated with *Pholiota squarrosa* (saprotrophic), *Laccaria bicolor* (ectomycorrhizal), both, or neither. We analysed growth, nitrogen uptake, and photosynthetic performance.

Ectomycorrhizal-inoculated plants showed greater growth, root development, and nitrogen accumulation than non-inoculated controls or those inoculated with saprotrophic fungi alone. Photosynthetic performance, particularly at 16 weeks, was also enhanced. In contrast, saprotrophic fungi increased nitrogen concentration in roots but did not improve plant biomass. Plant biomass and root architecture did not differ between EMF-only and dual-inoculated plants, suggesting that the addition of saprotrophic fungi did not further enhance or impair these traits. However, for nitrogen-related traits, dual-inoculated plants showed intermediate values between EMF-only and STF-only treatments. Despite these trends, statistical analysis did not detect a significant interaction between fungal guilds. These findings indicate that ectomycorrhizal fungi play a stronger role in promoting plant performance under nitrogen-limited conditions, likely through enhanced nutrient uptake and photosynthetic efficiency. Saprotrophic fungi alone did not promote plant growth under the experimental conditions, nor did their presence alter the benefits conferred by ectomycorrhizal fungi.

1. Introduction

Mycorrhizal and saprotrophic fungi are key players in plant nutrition in forest ecosystems, affecting nutrient availability and plant nutrient acquisition (Baldrián, 2017). Saprotrophic fungi (STF) decompose non-living organic matter, such as leaf litter, releasing carbon (C) and other essential nutrients back into the ecosystem. While the fungi primarily utilise the C for their own metabolism, nitrogen (N) and other nutrients may remain available in the soil, benefiting other organisms, such as plants and other soil fungi (e.g., mycorrhizal fungi) that can take up and use these resources (Setälä and McLean, 2004; Talbot et al., 2013). Ectomycorrhizal fungi (EMF) form associations with the roots of many plant species, enhancing nutrient uptake, particularly N and phosphorus, in exchange for C from photosynthesis (Smith and Read, 2010). This relationship is a type of symbiosis, and when both partners benefit, it is referred to as mutualism. However, mycorrhizal symbiosis exists along a continuum, ranging from mutually beneficial interactions to more unbalanced ones, where one partner may appropriate resources with limited return. The nature of the interaction can shift depending on environmental conditions and the physiological status of the partners (Pena et al., 2023; Pena and Tibbett, 2024).

In forests of the Northern Hemisphere, N is one of the most limiting nutrients for plant growth, largely because a significant portion of soil N is bound in decomposition-resistant organic forms that are not readily available to plants (Näsholm and Persson, 2001). To overcome this limitation, many tree species rely on EMF to acquire N (Read, 1991;

---

* Corresponding author at: Sustainable Land Management, School of Agriculture, Policy and Development, University of Reading, Reading, UK.
E-mail address: rodica.pena@reading.ac.uk (R. Pena).

https://doi.org/10.1016/j.envexpbot.2025.106246
Received 1 July 2025; Received in revised form 31 August 2025; Accepted 19 September 2025

Phillips et al., 2013). EMF enhance N uptake through extensive hyphal networks that explore the soil beyond the rhizosphere, increasing the plant's access to inorganic N sources (Tibbett and Sanders, 2002; Pena, 2016). Some EMF directly access N from soil organic substrates (Nicolás et al., 2019; Lindahl et al., 2021). However, their enzymatic abilities for decomposing organic substrates are generally lower than those of STF (Lindahl and Tunlid, 2015). Recent meta-analyses suggest that EMF can indirectly promote decomposition by stimulating STF activity, particularly under conditions such as a low substrate C:N ratio (Choreño-Parra and Treseder, 2024), low pH combined with a high C:N ratio (Fernandez and See, 2025; Pena et al., 2025) or in more fertile soils (Pena et al., 2025). This occurs through increasing the inputs of labile C, a process known as "priming" (Johansson et al., 2009; Fontaine et al., 2011; Zhang et al., 2019). In this way, EMF may also benefit from N released through enhanced organic matter decomposition (Pena et al., 2013b). In contrast, numerous studies have also shown that EMF suppress decomposition by competing with STF for N, a phenomenon referred to as the "Gadgil effect" (Gadgil and Gadgil, 1971; Averill et al., 2014; Fernandez and Kennedy, 2016). This effect likely depends in part on substrate quality, reflecting nutrient availability (Fernandez et al., 2020).

The interactions between STF and EMF guilds are particularly relevant under nutrient-limited conditions, where they may influence plant performance. While considerable progress has been made in identifying the ecological drivers of fungal interactions (Fernandez et al., 2020; Mayer et al., 2023; Fernandez and See, 2025; Pena et al., 2025) and in quantifying their influence on soil C cycling in complex forest ecosystems (Fernandez et al., 2020; Lang et al., 2021; Whalen et al., 2021; Carteron et al., 2022), their effects on plant performance remain largely unexplored. Competitive interactions between STF and EMF, particularly those that suppress EMF (Shaw et al., 1995; Zadworny et al., 2004) may impair plant performance (Pereira et al., 2012). Conversely, STF may also facilitate EMF functioning by translocating nutrients to the EMF mycelium (Lindahl et al., 1999, 2001), or promote plant growth more directly by enhancing nutrient cycling and improving soil health (Clocchiatti et al., 2020, 2021). Despite these insights, the direct impact of EMF-STF interactions on plant performance under nutrient-limited conditions remains largely untested. The study aimed to investigate whether such interactions influence plant growth under low-nutrient soils.

To achieve this, we used a plant-centric simplified experimental design that excluded the complexity of natural soils and microbial communities. This reduction in background variability provided a controlled setting in which the individual and combined effects of STF and EMF could be clearly isolated and evaluated. Micropropagated poplar (*Populus* × *canescens*) plantlets were grown under nutrient-poor conditions in the presence or absence of STF (*Pholiota squarrosa*), EMF (*Laccaria bicolor*), or both. The genus *Pholiota* includes wood and soil saprotrophs with a global distribution (Huang et al., 2024; Liu et al., 2024; He et al., 2025) and is commonly found in association with poplar and beech trees (Overholts, 1927; Lee et al., 2020). *L. bicolor* is a well-characterised EMF known to colonise poplar roots and enhance plant growth, primarily by improving N uptake (Lucic et al., 2008). The nutrient source consisted of a mixture of more recalcitrant beech and less recalcitrant poplar leaf litter.

Nitrogen is one of the essential elements for plant growth and development, as it is a key component of amino acids, proteins, and photosynthetic enzymes and pigments (Marschner, 2011; Mu and Chen, 2021). Nitrogen deficiency can severely limit photosynthesis by reducing both light absorption capacity and the efficiency of carbon fixation through carboxylation (Mu and Chen, 2021). Thus, we hypothesise that (1) EMF enhance plant N uptake and photosynthetic capacity; (2) STF promote plant growth by decomposing more recalcitrant leaf, leading to increased N availability for plant; and (3) EMF-STF interactions influence plant growth either positively, through facilitation or negatively, through competition for resources, ultimately affecting plant N nutrition.

2. Materials and methods

2.1. Plant and fungal material

Grey poplars (*Populus* x *canescens*: *Populus tremula* x *Populus alba*, clone INRA 717–1-B4) were multiplied by *in-vitro* micropropagation and co-cultivated with fungi in square Petri dishes (12 × 12 cm) using the cellophane membrane sandwich system described by Müller et al. (2013). The plantlets were initially grown for three weeks in Magenta ™ plant culture boxes (GA-7, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) on half-strength Murashige & Skoog (½MS) medium (Murashige and Skoog, 1962) solidified with Gelrite (Duchefa, Haarlem, Netherlands) in a plant incubator (FOC 200IL, Velp Scientifica, Usmate Velate, Italy) under controlled conditions: 21°C, 60 % relative humidity, and a 16-hour photoperiod. During that time, 22 plugs (about 5 mm diameter) of fungal strains *Laccaria bicolor* (strain S238N Maire P.D. Orton, originally provided by F. Martin, INRA, Nancy, France) and *Pholiota squarrosa* (obtained from stock collection Forest Botany, Göttingen, Germany), maintained in culture in darkness at 4°C on modified Melin-Norkrans (MMN) medium, were transferred to square Petri dishes on two halves of sterilised cellophane onto the MMN medium. The Petri dishes were then sealed with Parafilm (Sigma-Aldrich, Merck KGaA, Darmstadt) and incubated at 21°C for four weeks.

The poplar plantlets and fungi were then transferred together into new square Petri dishes filled with MMN_{low} medium (Müller et al., 2013), which differs from standard MMN by having 7.5 times less sugar and a slightly higher pH (5.8 vs. 5.2). In the sandwich system, agar medium was applied only to the lower half of the dish, covered with a cellophane membrane on which the poplar plantlet was placed. The fungal cellophane was then positioned directly on top, ensuring that the fungal mycelium made direct contact with the poplar roots between the two membranes. For non-inoculated control (NF) plants, sterile cellophane was used instead of fungal-inoculated cellophane. The lower halves of all Petri dishes were wrapped in aluminium foil to simulate dark soil conditions, and all dishes were incubated under the same controlled conditions (21°C, 60 % RH, 16 h light/day). After three weeks, once mycorrhization was visible, the plantlets were transferred to pots containing the growth substrate.

2.2. Plant cultivation

Poplar plantlets together with the fungal mycelium grown on the cellophane were removed from the MMN agar medium and transferred to 3 L (16 cm diameter) pots filled with a substrate composed of TerraGreen® clay (American Granules Plain, OIL-DRI, UK), silica quartz sand (Sibelco, UK) and medium-sized vermiculite (Sinclair, Cheshire, UK) in a 700:1600:100 ratio by mass. Prior to use, all components were heat-treated to reduce microbial loads: they were heated to 105°C for 1 h, allowed to rest for 24 h, and then reheated under the same conditions, as described by Soltangheisi et al. (2024). Following the final treatment, the soil was dried in an oven at 90°C, leaving a substrate disinfested of fungal propagules but not strictly sterile. Each pot was amended with 6.0 g of milled leaf litter powder of European beech (*Fagus sylvatica*) and Grey poplar in the ratio of 3:1, homogenously mixed into the substrate. To minimise microbial contamination, the leaf litter was dried at 90°C for two days prior to milling. To assess fungal presence, 200 μL of a substrate suspension (0.5 g of substrate mixed with 10 mL of sterile distilled water) was plated onto PDA and MMN agar. Plates were incubated at room temperature for 7 days, and no microbial growth was observed. For five pots per treatment, the poplar leaf litter was enriched with ^{15}N (4.96 ± 1.11 Atom % ^{15}N, 0.76 ± 0.06 % N). The beech litter originated from Rogate 718 intensive forest monitoring Level II site in South England (51°01'00"N, 0°52'01.0"W). Each plant was individually potted, resulting in four treatments, each with eight replicates: five plants received ^{15}N labeled litter and three received unlabelled litter. While cellophane in the sandwich system is typically used to limit fungal spread and facilitate mycelium transfer (Schreider et al., 2022), *P. Squamosa* could not be fully removed from the membrane for transfer into pots. The intended reference inoculum was the 11 fungal plugs (5 mm diameter) per plant, grown on cellophane membranes in the sandwich system. When mycelium could not be removed from the membrane, we substituted the missing material with an equivalent number of freshly excised fungal-agar plugs taken directly from *P. squarrosa* culture plates. To ensure consistency in agar volume between treatments, an equal number of agar-only plugs from *L. bicolor* cultures were added to the EMF treatments. In the non-inoculated (NF) control, sterile agar plugs were added in place of fungal material.

Plants were cultivated in a growth chamber (Controlled Environment Laboratory at the University of Reading) under controlled environmental conditions: 150 μmol $m^{-2}s^{-1}$ PAR with a 16-hour photoperiod, an air temperature of 22°C, and relative humidity maintained between 70–80 %. The cultivation period lasted 23 weeks. Pots were watered regularly with demineralised water (every three days with 300 mL). Gravimetric soil moisture content averaged 31.1 ± 0.61 %, with no significant differences between treatments ($P = 0.171$). The position of each plant was randomly assigned at the beginning of the experiment and rotated weekly to minimise positional effects.

To assess leaf litter decomposition, a mesh litterbag (1.3 mm mesh) containing 2.0 g of beech leaf litter was placed on the surface of the substrate in each pot.

2.3. *Plant growth, chlorophyll fluorescence, and gas exchange measurements*

Plant height was recorded weekly from the second week onward throughout the 23-week growth period in the controlled chamber. Chlorophyll fluorescence measurements were conducted on dark-adapted leaves at two time points using the integrated fluorometer of a portable photosynthesis system (LI-6800, LI-COR Biosciences, Lincoln, NE, USA).

Photosynthesis light response curves were recorded under a series of controlled light intensities (1500, 800, 200 and 50 μmol m^{-2} s^{-1} PPFD) between 09:00 and 14:00 on fully developed, healthy leaves (typically the 5th to 8th leaf from the apex). Measurement conditions were standardised across all treatments, with leaf temperature maintained at 24 °C, relative humidity around 50 %, a flow rate of 500 μmol s^{-1} and fan speed set to 10,000 rpm. The reference CO_2 concentration was set to 740 μmol mol^{-1} to minimise short-term CO_2 limitation and ensure stable photosynthetic responses. Gas exchange parameters, including net photosynthetic rate (A), stomatal conductance (g_s), and the intercellular-to-ambient CO_2 ratio (C_i/C_a), were extracted from light response curves at 800 μmol m^{-2} s^{-1} PPFD, corresponding to the light-saturation point where photosynthetic responses had stabilised (Pena and Tibbett, 2025). To assess potential differences in carboxylation efficiency, we calculated the A/C_i ratio as a proxy for photosynthetic capacity.

2.4. *Harvest*

At harvest, plant height was recorded, and stem diameter was measured 2 cm above the root collar using a digital calliper. Leaves, stems, and roots were separated and weighed individually to determine total fresh biomass. The number of leaves per plant was recorded, and all leaves were scanned using a flatbed scanner (Canon, UK) for subsequent image analysis. Leaves and stems were dried at 60°C to constant weight, and their dry biomass was determined. Roots were carefully washed with demineralised water, then separated into fine roots and coarse roots, weighed, wrapped in moist tissue paper, and stored at 4°C for further analysis.

Litterbags were carefully retrieved, gently opened, and the remaining leaf material was cleaned, dried, and weighed. Decomposition was calculated as the percentage of mass loss relative to the initial dry weight.

Within a few days, root systems were analysed using the WinRHIZO image analysis system (WinRHIZO, Regent Instruments Canada, Montreal, Canada) (Pena and Tibbett, 2025). A subset of approximately 500 root tips per plant was examined under a microscope (DM2700M, Leica Microsystems, Wetzlar, Germany) to assess ectomycorrhizal colonisation. Mycorrhization rate was calculated using the following formula:

EM colonisation (%) = (Number of mycorrhizal root tips / Number of total vital root tips) × 100

After analysis, the remaining root material was dried at 60°C to constant weight for dry biomass determination. For leaf area measurement, each leaf was measured from scanned pictures using ImageJ software (https://imagej.net/ImageJ).

Specific Leaf Area (SLA) was calculated for the entire plant: SLA = Leaf Area (cm^2)/ Leaf Dry Biomass (g).

2.5. *Soil nutrient analysis*

Plant-available nutrients were assessed using Plant Root Simulator (PRS®) probes (Western Ag Innovations Inc., Saskatoon, Canada), which consist of ion-exchange resin membranes embedded in plastic supports that mimic root uptake by adsorbing nutrient ions from the soil solution over time. Anion probes contain positively charged membranes that attract negatively charged ions such as nitrate (NO_3^-), phosphate ($H_2PO_4^-$, HPO_4^{2-}), and sulfate (SO_4^{2-}). Cation probes have negatively charged membranes that adsorb positively charged ions, including ammonium (NH_4^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). Probe pairs were inserted vertically into tubes containing the plant growth substrate at a 10 cm depth and left in place for 7 days to allow ion accumulation. The substrate, comprising both baseline (initial) samples and those collected at the end of the experiment from each treatment, was previously stored at −20 °C and was thawed prior to analysis.

2.6. *N stable isotope measurements*

For the measurements of N and ^{15}N, fine root tissues were ground into a fine powder using a Tissuelyser (TissuerLyser II, QIAGEN, Manchester UK). Total N and ^{15}N concentrations were determined using an isotope ratio mass spectrometer (IRMS) at the Hutton Institute, Craigiebuckler, Aberdeen, UK. The relative ^{15}N Atom percent excess (APE) in labelled samples was calculated by subtracting the natural abundance values from the measured enriched values. The total ^{15}N content was then calculated as biomass multiplied by percent N, multiplied by APE.

Leaf N content was measured from dried leaf samples that were ground to a fine powder using a TissueLyser II (Qiagen Ltd., Manchester, UK). Approximately 0.1 mg of each homogenised sample was weighed into tin capsules and analysed for total C and N content using a CHN628 Series elemental analyser (LECO Corporation, St. Joseph, MI, USA).

2.7. *Statistical analysis*

Statistical analyses conducted with R software version 4.2.2 (R Core Team, 2022). Prior data analysis, normal distribution and homogeneity of the variances were assessed visually by plotting the residuals or performing the Shapiro-Wilk test and Levene test. When data met assumptions of normality and equal variances, a one-way ANOVA was conducted to test for treatment effects. Post hoc comparisons were conducted using different methods depending on the analysis type. Tukey's HSD test was used for all pairwise comparisons between levels of a single factor (e.g., treatment groups) using *emmeans* package (Lenth, 2024). For analyses involving multiple contrasts or custom combinations of factor levels, we used the Sidak correction to adjust for multiple testing.

When assumptions were violated, a non-parametric Kruskal-Wallis test was used, followed by Dunn's post hoc tests with Holm adjustment for multiple comparisons (*FSA* package, Ogle et al., 2024). Grouping letters was assigned using the *rcompanion* package (Mangiafico, 2024).

Plant height data collected over time were analysed using a linear mixed-effects model to account for repeated measurements on the same individuals. The model included Treatment, Time Point, and their interaction as fixed effects, with Plant ID included as a random effect (*nlme* package, Pinheiro et al., 2023). The plots were generated using the *ggplot2* package (Wickham, 2016).

Analyses were conducted using complete-case data. Any observations with missing values for a given variable were excluded from that specific analysis.

3. Results

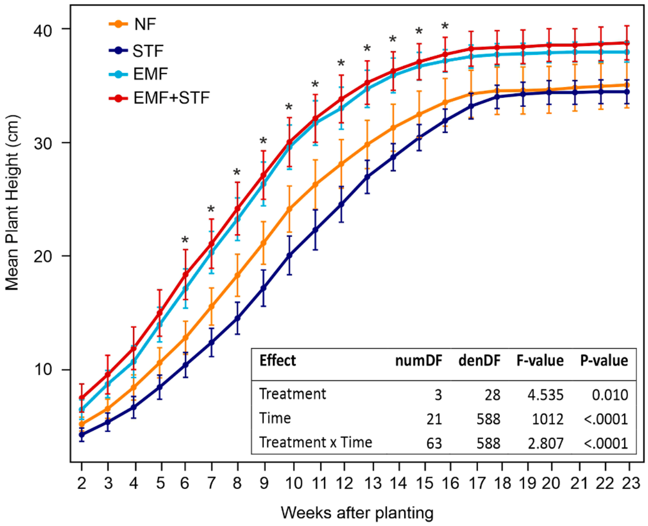
3.1. Influence of mycorrhizal and saprotrophic fungi on plant traits and biomass

We first examined whether the presence of fungi in the substrate and the formation of ectomycorrhizas with poplar roots changes the plant traits and performance compared to plants grown without fungal inoculation. After 23 weeks of growth in nutrient-poor substrate, about 7 % of root tips of poplars inoculated with *L. bicolor* formed ectomycorrhizas, whereas those of plants grown in the heat-treated substrate were non-mycorrhizal (Table 1). Leaf area and specific leaf area (SLA) were approximately 1.5–1.7 times greater in NF and STF plants compared to those inoculated with EMF or EMF+STF ($P = 0.006$ and $P < 0.001$, respectively). Leaf biomass did not differ significantly among treatments (Table 1). The stem diameter and stem biomass were larger in plants inoculated with both EMF and STF fungi compared to those inoculated with STF alone (Table 2). STF plants also exhibited the lowest coarse and fine root biomass among all treatments (Table 1). The highest fine root biomass was observed in EMF-inoculated plants, particularly when STF fungi were absent and only EMF was added to the substrate. This treatment also resulted in the highest root-to-shoot ratio (Table 1). Whole-plant biomass was lowest in STF plants, being approximately 1.4 times lower than in EMF-inoculated plants ($P = 0.007$, Table 1).

A repeated measures ANOVA was conducted to examine the effects of treatment and time on plant height. There was a significant main effects of Treatment ($F_{(3,\ 28)} = 4.53$, $P = 0.010$) and Time ($F_{(21,\ 588)} = 1012$, $P < 0.001$), as well as a significant Treatment × Time interaction ($F_{(63,\ 588)} = 2.80$, $P = 0.001$), indicating that plant height varied over time and differed among treatments (Fig. 1). EMF+STF-treated plants were significantly taller than STF-treated plants between weeks 6 and 16, while EMF-treated plants were taller than STF-treated plants between weeks 7 and 16 (Fig. 1). However, these differences diminished by week 17, when plant height reached a plateau and no significant differences were observed among treatments (Fig. 1). The presence of EMF was generally beneficial for plant height, although the difference between EMF and NF plants was not statistically significant. NF plants tended to be shorter than EMF plants, but this trend did not reach significance (Fig. 1).

Table 2
Maximum quantum efficiency of PSII (Fv/Fm) measured when plants were 10 weeks and 23 weeks old. Values are means ± SE, (n = 8). Stars indicate significant differences between the two time points ($P \leq 0.05$), based on two-way ANOVA followed by Tukey's HSD test.

Treatment	Weeks	Fv/Fm
NF	10	0.756 ± 0.01*
	23	0.718 ± 0.01
STF	10	0.749 ± 0.01*
	23	0.712 ± 0.02
EMF	10	0.753 ± 0.00*
	23	0.711 ± 0.02
EMF+STF	10	0.742 ± 0.01
	23	0.719 ± 0.01



Effect	numDF	denDF	F-value	P-value
Treatment	3	28	4.535	0.010
Time	21	588	1012	<.0001
Treatment x Time	63	588	2.807	<.0001

Fig. 1. Plant height over time, measured weekly after planting. Plants which were not inoculated with fungi (NF), inoculated with ectomycorrhizal fungi (EMF), with saprotrophic fungi (STF), and a mixture of both EMF and STF fungi (EMF+STF). Data represent mean values ± SE (n = 8). Differences among treatments at each time point are marked with stars. A summary of the repeated measures ANOVA results is shown in the bottom-right inset.

Root surface area and the number of root forks (i.e., branching points) were approximately 1.4 times greater in EMF-inoculated plants compared to STF plants ($P = 0.026$ and $P = 0.005$, respectively; Fig. 2). Plants inoculated with both fungi, as well as NF plants, showed intermediate values between the EMF and STF treatments. No significant differences among treatments were observed for other fine root architectural traits, including total root length, specific root length, average

Table 1
Plant traits and biomass for four fungal treatments: non-inoculated (NF), inoculated with ectomycorrhizal fungi (EMF), saprotrophic fungi (STF), or a mixture of both (EMF+STF). Values are means ± SE, (n = 8). Different letters indicate significant differences between treatments ($P \leq 0.05$). EMF colonisation, Leaf area, and Specific leaf area were analysed using Kruskal–Wallis tests followed by Dunn's post-hoc test. All other variables were tested using one-way ANOVA with Sidak-adjusted post hoc tests.

Variables	NF	STF	EMF	EMF+STF
EMF colonisation (%)	0	0	7.39 ± 1.29 a	6.93 ± 1.38 a
Leaf Area (cm^2 plant^{-1})	248.12 ± 29.34 b	218.25 ± 33.29 ab	139.62 ± 11.41 a	135.88 ± 10.97 a
Stem Diameter (mm)	4.84 ± 0.22 ab	4.41 ± 0.22 a	5.13 ± 0.22 ab	5.25 ± 0.22 b
Stem dry mass (g)	1.71 ± 0.17 ab	1.51 ± 0.17 a	1.98 ± 0.17 ab	2.23 ± 0.17 b
Leaf dry mass (g)	1.14 ± 0.10 a	1.29 ± 0.10 a	1.11 ± 0.10 a	1.07 ± 0.10 a
Coarse root dry mass (g)	3.37 ± 0.35 ab	2.56 ± 0.35 a	4.22 ± 0.35 b	4.00 ± 0.35 b
Fine root dry mass (g)	3.78 ± 0.36 a	3.37 ± 0.36 a	5.7 ± 0.36 b	4.66 ± 0.36 ab
Plant dry mass (g)	10.01 ± 0.88 ab	8.72 ± 0.88 a	13.02 ± 0.88 b	11.96 ± 0.88 ab
Specific leaf area (cm^2 g^{-1})	231.54 ± 35.54 b	170.11 ± 22.48 b	126.03 ± 2.47 a	127.25 ± 1.81 a
Root-to-Shoot ratio	2.55 ± 0.12 ab	2.14 ± 0.12 a	3.22 ± 0.12 c	2.65 ± 0.12 b

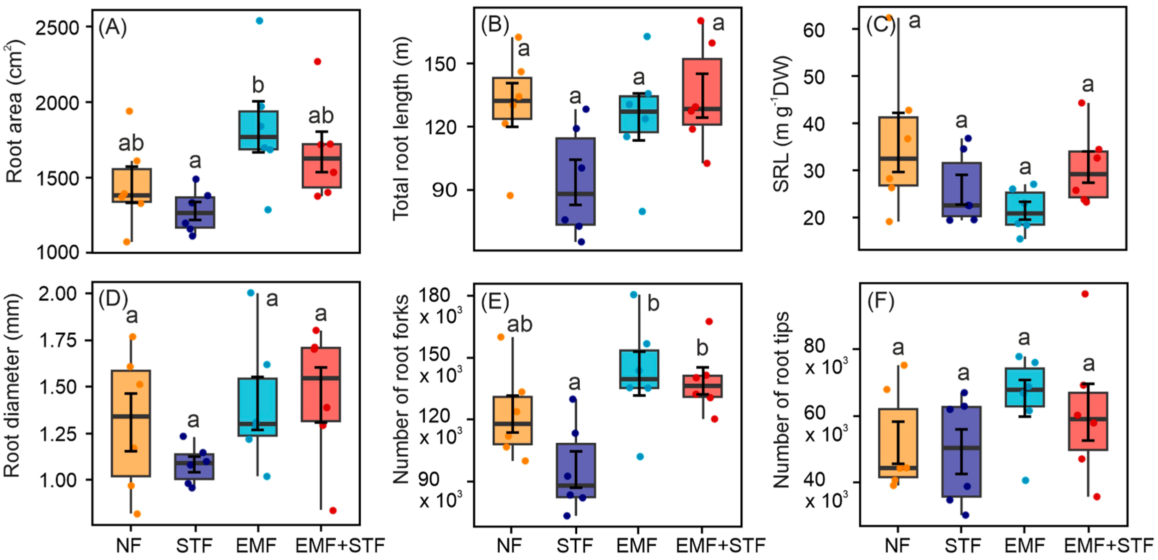


Fig. 2. Root architecture traits under different fungal treatments in poplar plants: non-inoculated (NF), inoculated with saprotrophic fungi (STF), ectomycorrhizal fungi (EMF), or a mixture of both (EMF+STF). (A) Fine root area; (B) Fine root total length; (C) Specific root length (SRL); and (D) Average root diameter, (E) Number of root forks, and (F) Number of root tips. Box plots show the distribution of individual values (dots), with horizontal lines indicating the median. Mean values ± standard error (SE) are shown as overlaid black points with error bars (n = 8). Different letters indicate significant differences among treatments ($P \leq 0.05$), which were determined using two-way ANOVA followed by Tukey's HSD test.

root diameter, and number of tips (Fig. 2).

3.2. Fungal influence on fine root nitrogen acquisition

Nutrient availability in the growth substrate changed substantially during the experiment compared to the initial (baseline) substrate. NO_3^-

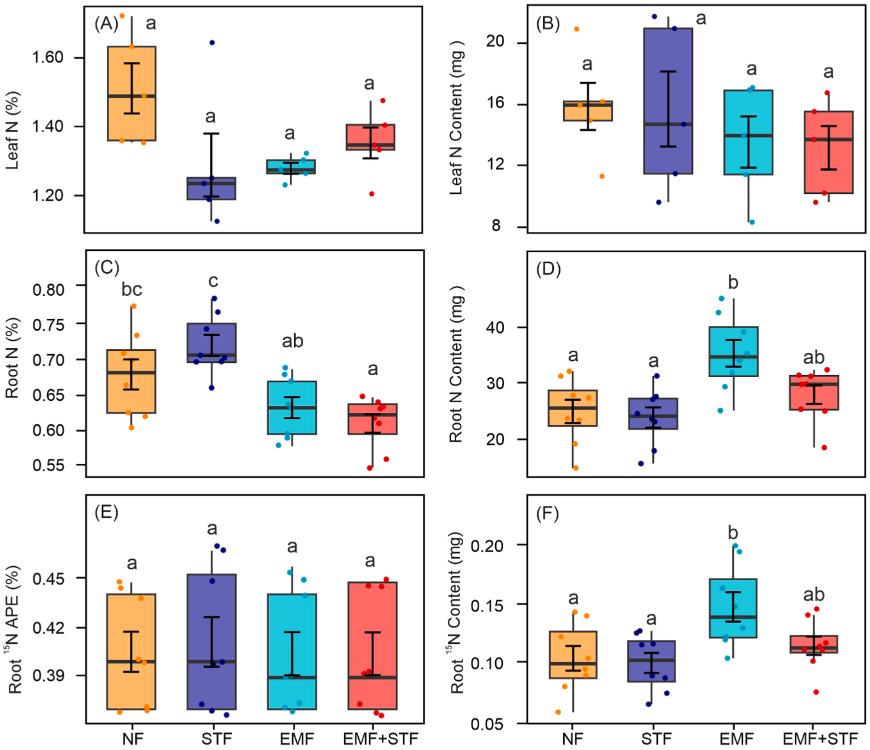


Fig. 3. Nitrogen concentration and isotopic composition of poplar fine roots under different fungal treatments: non-inoculated (NF), inoculated with saprotrophic fungi (STF), ectomycorrhizal fungi (EMF), or both (EMF+STF). (A) Leaf nitrogen concentration; (B) Total nitrogen content in leaves; (C) Root nitrogen concentration; (D) Total nitrogen content in roots; (E) Roots ^{15}N enrichment expressed as Atom Percent Excess (APE%); (F) Roots Excess ^{15}N content, calculated using APE% and total nitrogen content. Box plots show the distribution of individual values (dots), with horizontal lines indicating the median. Mean values ± standard error (SE) are shown as overlaid black points with error bars (n = 5 in A, B, E and F; n = 8 in C and D). Different letters indicate significant differences among treatments ($P \leq 0.05$). ^{15}N APE was analysed using Kruskal–Wallis tests followed by Dunn's post-hoc test. All other variables were analysed using two-way ANOVA followed by Tukey's HSD test.

and NH_4^+ levels declined below detection limits across all planted treatments, suggesting strong uptake by plants and microorganisms. Phosphorus and K availabilities also decreased during cultivation, while Ca availability increased relative to the baseline. No significant differences in nutrient availability were detected among the fungal inoculation treatments (Table S1).

Mass loss from the leaf litter bags averaged 18.41 ± 1.01 % with no significant differences between treatments ($P = 0.557$, data not shown).

Leaf N concentrations and total nitrogen contents did not differ among treatments (Figs. 3A, 3B). In fine roots, N concentrations were highest in STF-inoculated plants (0.72 ± 0.02 %), followed by NF plants (Fig. 3C). In contrast, EMF and EMF+STF treatments exhibited significantly lower N concentrations than STF, with values of 0.63 ± 0.02 % and 0.61 ± 0.02 %, respectively (Fig. 3C). There was no significant interaction between EMF and STF on N concentration ($F_{(1,28)} = 3.71$, $P = 0.064$; Fig. 3C). Despite lower N concentrations, total N fine root content was highest in EMF-inoculated plants due to their greater root biomass (Fig. 3D). EMF inoculation alone increased N content in the fine roots by approximately 1.4 times compared to NF and STF plants ($F_{(1,28)} = 12.95$, $P = 0.001$). STF alone did not significantly affect N content (Fig. 3D). The EMF+STF combination resulted in intermediate values between EMF and STF treatments, with no apparent interaction between EMF and STF on N content ($F_{(1,28)} = 2.44$, $P = 0.129$; Fig. 3D). No significant differences in ^{15}N APE and excess ^{15}N content were observed across treatments, with all groups showing similar medians of approximately 0.076 % (Fig. 3E) and 0.022 mg, respectively (Fig. 3F). However, in non-^{15}N labelled plants, EMF-inoculated plants showed higher natural content of ^{15}N than NF plants in the fine roots (Fig. S1B). This resulted in a 1.5 times larger ^{15}N accumulation in EMF and EMF+STF than NF and STF ^{15}N-labelled plants ($F_{(1,28)} = 7.26$, $P = 0.0118$, Fig. S1D).

3.3. *Fluorescence responses and photosynthetic performance to fungal inoculation*

The maximum quantum efficiency of PSII (Fv/Fm) was below the theoretical optimum of 0.80 in all treatments at all measuring times (Table S2), suggesting that all plants experienced a mild photoinhibition or environmental stress. There were no differences among treatments ($F_{(3,\ 56)} = 0.14$, $P = 0.936$), but there was a significant decline of Fv/Fm over time in most treatments ($F_{(1,\ 56)} = 15.38$, $P < 0.001$, Table 2), with the exception of the EMF+STF ($P = 0.202$). There was no significant interaction between treatment and time ($F_{(3,\ 56)} = 0.215$, $P = 0.885$; Table 2).

Photosynthetic light-response curves showed similar overall shapes across treatments, with no differences in the net photosynthetic rate (A) or light saturation point (Fig. S2). However, at 800 μmol $m^{-2}s^{-1}$ PPFD light intensity that falls within the plateau phase of the curves for all treatments (Fig. S2), comparison among treatments revealed differences in A at specific time points. At 8 weeks of age, NF plants showed higher A compared to other treatments, although the difference was statistically significant only when compared to STF-inoculated plants ($P = 0.039$, Fig. 4). By 16 weeks, EMF-inoculated plants exhibited a 1.5-fold higher A than NF plants ($P = 0.015$, Fig. 4).

Stomatal conductance was significantly higher in EMF-inoculated plants at 16 weeks of age, showing values 1.8 times greater than NF plants ($P = 0.001$) and 1.5 times greater than STF plants ($P = 0.014$), with no significant differences observed at other time points (Fig. S3). The Ci/Ca ratio, reflecting the balance between CO_2 supply and assimilation, ranged from 0.6 to 0.8 across treatments, indicating generally well-balanced internal CO_2 conditions. However, Ci/Ca was higher in EMF plants compared to NF ($P = 0.022$) and STF plants ($P = 0.021$; Fig. S4). To further assess carboxylation efficiency, we calculated the A/Ci ratio, which did not differ among treatments (Fig. S5), suggesting that differences in biochemical fixation capacity did not drive the observed variation in A.

4. Discussion

Although the influence of EMF-STF interactions on nutrient cycling in forest soils has been largely documented in recent years (Fernandez et al., 2020; Lang et al., 2021; Whalen et al., 2021; Carteron et al., 2022; Mayer et al., 2023; Fernandez and See, 2025), information on their effects on plant performance is scarce. Our results demonstrate that EMF and STF, individually and in combination, exert distinct effects on plant growth, root architecture, and N acquisition in nutrient-poor conditions.

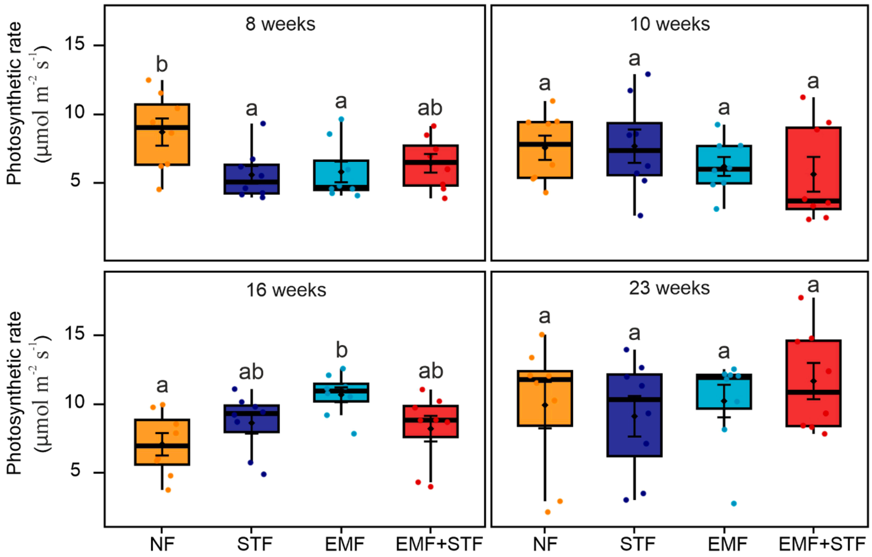


Fig. 4. Net photosynthetic rate (A) of poplar plants under different fungal treatments: non-inoculated (NF), inoculated with saprotrophic fungi (STF), ectomycorrhizal fungi (EMF), or both (EMF+STF). A was extracted from light response curves at a PPFD of 800 μmol m^{-2} s^{-1} at four developmental stages: 8, 10, 16, and 23 weeks after planting. Box plots show the distribution of individual values (dots), with horizontal lines indicating the median. Mean values ± standard error (SE) are shown as overlaid black points with error bars (n = 8). Different letters indicate significant differences among treatments ($P \leq 0.05$). Data from weeks 16 and 23 were analysed using Kruskal–Wallis tests followed by Dunn's post-hoc test. All other variables were analysed using two-way ANOVA followed by Tukey's HSD test.

4.1. Fungal inoculation modifies juvenile poplar traits and growth

The plant height data followed a biphasic growth pattern, characterised by a steady increase until week 17, followed by a plateau phase that persisted through to the final harvest at week 23. This shift likely reflects the onset of resource limitation (Fang et al., 2008). Plants were grown in a sandy, nutrient-poor substrate supplemented with a mixture of beech and poplar leaf litter as the sole nutrient source. Nitrogen content of the leaf litter was relatively low, ranging from 0.9 % to 1.5 % for beech (Zeller et al., 2000; Jacob et al., 2009) and averaging 0.8 % for poplar. Despite fungal inoculation, mass loss from the beech leaf litter remained close to 20 % across all treatments. In the substrate, by the end of the experiment, plant-available N forms (i.e, NO_3^- and NH_4^+) were nearly undetectable, while other macronutrients such as P and K were also markedly reduced. These results suggest that while early growth was supported by soluble compounds from the litter, the nutrient supply became progressively limiting over time, constraining further height gain during the later stages of the experiment. We found that inoculation with EMF, either alone or in combination with STF, significantly increased tree height between weeks 6 and 17, compared to non-inoculated plants or those inoculated with STF alone. However, by the end of the experiment, differences in total plant biomass between EMF and non-inoculated plants were no longer apparent. These findings are consistent with previous studies on young tree seedlings, which have reported enhanced growth and biomass accumulation in the presence of EMF under nutrient-limited conditions (Kennedy and Peay, 2007; Langenfeld-Heyser et al., 2007). However, several studies have also reported no significant growth differences, or even reduced growth, in EMF plants compared with non-mycorrhizal plants (Pena et al., 2013a; Shi et al., 2024). This variability is often attributed to the high C cost incurred by the host plant to sustain the fungal symbionts and to support the development of a more extensive root system that may be detrimental to plant investment in biomass (Verlinden et al., 2018). In our experiment, the mycorrhization rate reached only 7 %, which is relatively low compared to the 50 % colonisation reported by Shi et al. (2024) in a study involving *L. bicolor* and *Poplar* x *canescens*. However, low levels of ectomycorrhizal colonisation are not uncommon in juvenile plants (Nguyen et al., 2017; Köhler et al., 2018), likely due to the high associated C costs that may exceed the C budget of young, developing plants (Hobbie, 2006; Nehls et al., 2010).

The biomass of EMF plants was higher than that of STF plants, primarily due to increased root biomass. While the total number of root tips did not differ among treatments, EMF-inoculated plants promoted increased root branching, showing a significantly higher number of root forks than STF plants. This is consistent with previous reports showing that EMF can induce specific modifications in root architecture, including enhanced lateral branching (Ditengou et al., 2015). Increased fork numbers with stable tip numbers in EMF plants indicate a shift in root architectural complexity rather than an increase in overall root production, which was reflected by no difference in other root parameters. This may reflect a denser or more highly branched root network in the EMF treatment, potentially enhancing soil exploration and increasing surface area for nutrient uptake that was not necessarily needed in the STF treatment when the nutrients could be more available due to STF decomposing activity. The stability in tip number may also suggest, under nutrient scarcity, that the development of new root apices was not strongly affected, but rather that existing roots underwent more frequent lateral branching. Such architectural changes are consistent with EMF-driven modifications of root systems, where C allocation to fungal partners can be linked to altered root foraging strategies that favour intensive soil resource exploitation in localised zones rather than extensive new root tip formation (Chen et al., 2016).

Contrary to our second hypothesis, STF alone did not enhance plant biomass. This finding is notable, as it challenges the common assumption that STF universally support plant growth by accelerating organic matter decomposition and nutrient release (Clocchiatti et al., 2020). Instead, it aligns with previous observations of no differences in growth between non-inoculated and STF-inoculated *Castanea sativa* plants (Pereira et al., 2012). One possible explanation is that the contribution of *P. squarrosa* to nutrient release was insufficient to meet the plant demand under the given conditions. The observed lack of difference in litter decomposition across treatments further supports the idea that STF activity was not significantly stimulated under these conditions. It is possible that the controlled setting used here constrained the expression of STF functional potential, either due to imbalanced soil nutrient stoichiometry, as predicted by ecological stoichiometry theory (Zechmeister-Boltenstern et al., 2015) or due to the absence of a home-field advantage. The latter, a characteristic of efficient decomposer communities particularly under nutrient-poor conditions, refers to the enhanced decomposition of local species litter by locally adapted microbial communities (Veen et al., 2015; Benito-Carnero et al., 2021). It is important to note that the leaf litter was heat-treated prior to its use in substrate mixing or leaf litter bags to reduce microbial load. While this step is necessary, it may also lead to partial degradation of key structural components such as pectin, hemicellulose, cellulose, and lignin, compounds that are central to fungal decomposition dynamics. In particular, thermal degradation around 80–90°C affects pectin and hemicelluloses (Bufacchi et al., 2020) that are the most rapidly degraded constituents during the early stages of litter decomposition (Šnajdr et al., 2011). Additionally, heating may alter the profile of volatile organic compounds (VOCs) emitted from degrading leaf litter, which influences soil microbial communities (McBride et al., 2020). However, abiotic sources of VOCs, particularly hydrocarbons (e.g., methane, ethane, ethene, propene, and aromatic compounds like benzene and toluene), whose emissions increase with temperature, are generally less significant than biotic sources (Gray et al., 2010). Moreover, Derendorp et al., (2010) have shown that after heat-induced depletion, VOC formation can be initiated again when oxygen is present. Therefore, while drying leaf litter at 90°C may alter some VOC profiles, this treatment is unlikely to substantially impair fungal decomposition processes or microbial interactions, especially considering the resumption of VOC activity under aerobic conditions.

We also found that, contrary to our hypothesis, dual EMF-STF inoculation produced similar effects on plant biomass and root architecture as EMF-only inoculation. This suggests that the presence of EMF did not induce a priming effect that enhanced STF performance relative to STF-only treatments, as might have been expected (Zhang et al., 2019). Nor did STF outcompete EMF and suppress its beneficial effects, a scenario observed by Pereira et al., (2012) and reflected in numerous culture-based studies reporting antagonistic interactions between EMF and STF (Shaw et al., 1995; Leake et al., 2001; Zadworny et al., 2004). Similar outcomes were reported by (Albrechtová et al., 2012), who found that dual inoculation with STF and arbuscular mycorrhizal fungi enhanced plant growth to a similar extent as mycorrhizal-only treatments. Although their study focused on a cultivated vegetable species and involved arbuscular rather than EMF, their findings lend additional support to the interpretation that dual fungal inoculations do not always result in additive or synergistic effects as we have hypothesised.

4.2. Greater leaf litter-derived N in the fine roots of ectomycorrhizal-inoculated plants

The low-nutrient substrate was supplemented with poplar and beech leaf litter as a source of additional nutrients. We assessed N acquisition by measuring total N and ^{15}N concentrations and contents in fine roots, using ^{15}N as a tracer for N specifically derived from the poplar leaf litter. The two litter types differ in decomposability, which can influence microbial access to labile versus recalcitrant organic matter (Cornwell et al., 2008). Compared to poplar, beech leaf litter has higher lignin content and C:N ratios, leading to slower mass loss and reduced rates of nutrient mineralisation under similar environmental conditions (Berg and McClaugherty, 2014). We observed no differences in N acquisition from the ^{15}N-labelled poplar leaf litter across fungal treatments. However, STF-inoculated plants exhibited the highest N concentration. Although this may be attributed to the functional capacity of *P. squarrosa*, a wood-decomposing fungus (Miller Jr. et al., 1996) with strong saprotrophic capabilities, in mobilising N from the more recalcitrant beech leaf litter into the soil, the mass loss of the beech leaf litter bags was consistent across treatments, suggesting that STF-inoculated plants may not have benefited from overall litter decomposition. Thus, the higher N concentration may reflect enhanced access to labile N compounds released early in the decomposition process, rather than the STF influence on mineralisation of bulk litter. This is supported by the similar N levels in STF-inoculated plants and non-inoculated plants, suggesting that both groups primarily accessed the same pool of readily available N. Such uptake is consistent with the ability of plants to absorb soluble organic N compounds-such as amino acids and small peptides, released during early-stage litter breakdown, without requiring microbial mineralisation (Näsholm et al., 2009).

Nitrogen concentrations were lower in the fine roots of EMF-inoculated plants, both when inoculated alone and in combination with STF. However, this indicates no competition for access to the labile N compounds between EMF and host plants that could occur under strong N limitation (Näsholm et al., 2013; Pena and Polle, 2014), as N content was higher in EMF-inoculated plants compared to both non-inoculated and STF-inoculated plants, likely due to their greater root biomass. Similar patterns of increased total N and other nutrients in EMF-inoculated plants relative to STF-inoculated ones have also been reported by Pereira et al. (2012), further supporting this interpretation. Again, the EMF effect was not found for the ^{15}N content, which did not vary among treatments, indicating access to the beech leaf litter-originated N. Nitrogen mobilisation from beech leaf litter decomposition may be possible to a limited extent. Although *L. bicolor* has lost many genes encoding the enzymatic machinery required to degrade recalcitrant lignocellulosic substrates (Kohler et al., 2015), it does express a range of oxidoreductases that are thought to contribute to N mobilisation through the partial decomposition of organic matter (Shah et al., 2016). However, the similar decomposition rates of beech leaf litter across treatments suggest that the observed differences are more likely due to the greater efficiency of EMF fungi in nutrient scavenging, rather than an increase in overall decomposition rate per se (Pena et al., 2013b; Shah et al., 2016). This perspective is further supported by the positive correlation between the number of root forks and root N content (data not shown), indicating that more extensive EMF plant roots may have facilitated enhanced nutrient acquisition.

The ^{15}N natural abundance, measured in the fine roots of non-labelled plants, was similar among treatments, indicating a similar source of N (Deb et al., 2024). During decomposition, N released from organic matter is often enriched in ^{15}N due to fungal fractionation processes (Craine et al., 2015). Thus, plants absorbing N from those sources may exhibit higher ^{15}N enrichments. In contrast, ectomycorrhizal plants often show ^{15}N depletion relative to their N source, as EMF preferentially retain ^{15}N and transfer ^{14}N to their hosts (Hobbie and Högberg, 2012).

The interactions between EMF and STF fungi in the mixed inoculation treatment were not statistically significant, providing no evidence for either facilitation or competition between the two fungal groups. This outcome contradicts our third hypothesis, which anticipated interactive effects, either synergistic or antagonistic, on plant N acquisition. However, the nature of EMF-STF interactions is known to span a facilitation-competition continuum, and recent studies have reported neutral outcomes where no clear interaction occurs (Pena et al., 2025). For instance, Pereira et al., (2012) found that the outcome of dual inoculation depended on the timing of STF introduction: competition was observed when both fungi were introduced simultaneously, but not when EMF had time to establish before STF inoculation. We may speculate that the lack of interaction observed in our study may reflect a context-dependent neutrality rather than a true absence of functional interplay.

4.3. Enhanced photosynthetic performance in ectomycorrhizal-inoculated plants

Although growth differences were apparent among treatments, the health and functionality of the photosynthetic apparatus, as indicated by values of Fv/Fm in dark-adapted leaves, were similar for all plants. The Fv/Fm values were slightly below the theoretical maximum of 0.83, indicating a mild level of chronic photoinhibition or stress across all treatments (Maxwell and Johnson, 2000). This reduction in PSII efficiency is likely linked to nutrient-related constraints (Warren, 2006; Urban et al., 2017), particularly under controlled mesocosm conditions when other potentially stressful factors were controlled. Numerous studies have reported significant declines in Fv/Fm under nutrient-limited conditions in trees and seedlings (Grassi and Bagnaresi, 2001). More specifically, N deficiency has been shown to reduce Fv/Fm, reflecting impaired photosynthetic performance (Mu and Chen, 2021). Nevertheless, the 16-week-old plants inoculated with EMF exhibited significantly higher rates of net CO_2 assimilation (A), stomatal conductance (g_s), and intercellular-to-ambient CO_2 ratio (C_i/C_a) compared to non-mycorrhizal plants. No significant differences were observed in the quantum yield of CO_2 assimilation in light-adapted leaves (ΦCO_2) across treatments (data not shown). The light intensity during measurements (800 μmol $m^{-2}s^{-1}$) was saturating and non-limiting, ensuring that differences in A were not due to variation in light availability. It is important to note that the gas exchange measurements were conducted at an elevated reference CO_2 (740 μmol mol^{-1}), which reduced short-term CO_2 limitation and supported stabilised photosynthetic responses. While this elevated CO_2 level may have enhanced A and reduced g_s relative to ambient conditions, all measurements were conducted under the same conditions, allowing robust comparisons among treatments. The lack of variation in ΦCO_2 and Fv/Fm implies that light-use efficiency remained stable across treatments, suggesting that differences in A were primarily driven by enhanced CO2 acquisition capacity, as indicated by higher gs and a higher C_i/C_a ratio. A role for internal conductance, such as mesophyll conductance (g_m), cannot be excluded, though the similarity of A/Ci and ΦCO_2 among treatments is consistent with g_m not differing substantially. Furthermore, the lack of variation in the A/C_i ratio indicates that biochemical limitations (e.g., Rubisco activity) were not the primary determinant of A. These findings suggest that the physiological advantage conferred by mycorrhizal symbiosis under nutrient-limited conditions was primarily due to improved resource uptake and CO_2 availability, rather than enhanced biochemical assimilation capacity (Shi et al., 2024).

These findings indicate that EMF play a more prominent role in enhancing plant performance under nutrient-limited conditions, likely by modifying root architecture, improving N uptake, and increasing photosynthetic efficiency. However, it is important to acknowledge the limitations of our simplified experimental setup, which lacked key soil biotic components such as invertebrates that are known to significantly influence fungal-mediated nutrient mineralisation and distribution (Crowther et al., 2012). Additionally, the use of only one representative fungus from each functional guild does not capture the high functional and ecological diversity of natural fungal communities (Pena and Polle, 2014; Schröter et al., 2018; Wutkowska et al., 2021). Future studies incorporating more complex soil ecosystems and diverse fungal assemblages are needed to determine whether these patterns persist under more variable and field-realistic conditions.

CRediT authorship contribution statement

Mark Tibbett: Writing – review & editing, Project administration, Funding acquisition. **Milner Gemma Lou:** Investigation. **Rodica Pena:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation,

Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

We thank Liam Doherty for his assistance in organising the experimental growth chambers, and Emanuel Scherdien Da Rosa for coordinating the shipping of samples. We also thank Prof. Andrea Polle for kindly providing *Populus* cuttings and for valuable discussions. The work has been funded by the Natural Environment Research Council – UKRI (NE/W005816/1).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2025.106246.

References

Albrechtová, J., Látr, A., Nedorost, Ľ., Pokluda, R., Posta, K., Vosátka, M., 2012. Dual inoculation with mycorrhizal and saprotrophic fungi applicable in sustainable cultivation improves the yield and nutritive value of onion. Sci. World J. 2012, 374091.

Averill, C., Turner, B.L., Finzi, A.C., 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505, 543–545.

Baldrián, P., 2017. Microbial activity and the dynamics of ecosystem processes in forest soils. Curr. Opin. Microbiol. 37.

Benito-Carnero, G., Gartzia-Bengoetxea, N., Arias-González, A., Rousk, J., 2021. Low-quality carbon and lack of nutrients result in a stronger fungal than bacterial home-field advantage during the decomposition of leaf litter. Funct. Ecol. 35, 1783–1796.

Berg, B., McClaugherty, C., 2014. Plant litter: decomposition, humus formation, carbon sequestration. Springer-Verlag, Berlin Heidelberg.

Bufacchi, P., Bizzo, W.A., Buckeridge, M.S., Franco-Jacome, D.L., Grandis, A., Cambler, A.B., Krieger Filho, G.C., 2020. Thermal degradation of leaves from the Amazon rainforest litter considering non-structural, structural carbohydrates and lignin composition. Bioresour. Technol. Rep. 11, 100490.

Carteron, A., Cichonski, F., Laliberté, E., 2022. Ectomycorrhizal stands accelerate decomposition to a greater extent than arbuscular mycorrhizal stands in a Northern deciduous forest. Ecosystems 25, 1234–1248.

Chen, W., Koide, R.T., Adams, T.S., DeForest, J.L., Cheng, L., Eissenstat, D.M., 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. Proc. Natl. Acad. Sci. USA 113, 8741–8746.

Choreño-Parra, E.M., Treseder, K.K., 2024. Mycorrhizal fungi modify decomposition: a meta-analysis. N. Phytol. 242, 2763–2774.

Clocchiatti, A., Hannula, S.E., Hundscheid, M.P.J., klein Gunnewiek, P.J.A., de Boer, W., 2021. Stimulated saprotrophic fungi in arable soil extend their activity to the rhizosphere and root microbiomes of crop seedlings. Environ. Microbiol. 23, 6056–6073.

Clocchiatti, A., Hannula, S.E., van den Berg, M., Korthals, G., de Boer, W., 2020. The hidden potential of saprotrophic fungi in arable soil: patterns of short-term stimulation by organic amendments. Appl. Soil Ecol. 147, 103434.

R. Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL ⟨https://www.R-project.org/⟩.

Cornwell, W.K., Cornelissen, J.H.C., Amatangelo, K., et al., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11, 1065–1071.

Craine, J.M., Brookshire, E.N.J., Cramer, M.D., Hasselquist, N.J., Koba, K., Marin-Spiotta, E., Wang, L., 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. Plant Soil 396, 1–26.

Crowther, T.W., Boddy, L., Hefin Jones, T., 2012. Functional and ecological consequences of saprotrophic fungus–grazer interactions. ISME J. 6, 1992–2001.

Deb, S., Lewicka-Szczebak, D., Rohe, L., 2024. Microbial nitrogen transformations tracked by natural abundance isotope studies and microbiological methods: a review. Sci. Total Environ. 926, 172073.

Derendorp, L., Holzinger, R., Wishkerman, A., Keppler, F., Röckmann, T., 2010. VOC emissions from dry leaf litter and their dependence on temperature. Biogeosciences Discuss. 7.

Ditengou, F.A., Müller, A., Rosenkranz, M., Felten, J., Lasok, H., van Doorn, M.M., Legué, V., Palme, K., Schnitzler, J.-P., Polle, A., 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. Nat. Commun. 6, 6279.

Fang, S., Xie, B., Liu, J., 2008. Soil nutrient availability, poplar growth and biomass production on degraded agricultural soil under fresh grass mulch. For. Ecol. Manag. 255, 1802–1809.

Fernandez, C.W., Kennedy, P.G., 2016. Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? N. Phytol. 209, 1382–1394.

Fernandez, C.W., See, C.R., Kennedy, P.G., 2020. Decelerated carbon cycling by ectomycorrhizal fungi is controlled by substrate quality and community composition. N. Phytol. 226, 569–582.

Fernandez, C.W., See, C.R., 2025. The ph influence on ectomycorrhizal nitrogen acquisition and decomposition. N. Phytol. 246, 867–875.

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. 43, 86–96.

Gadgil, R.L., Gadgil, P.D., 1971. Mycorrhiza and litter decomposition. Nature 233, 133.

Grassi, G., Bagnaresi, U., 2001. Foliar morphological and physiological plasticity in Picea abies and Abies alba saplings along a natural light gradient. Tree Physiol 21 (12–13), 959–967. https://doi.org/10.1093/treephys/21.12-13.959.

Gray, C.M., Monson, R.K., Fierer, N., 2010. Emissions of volatile organic compounds during the decomposition of plant litter. J. Geophys. Res. Biogeosciences 115.

He, Y., Liu, B., Ouyang, X., et al., 2025. Whole-Genome sequencing and fine map analysis of pholiota nameko. J. Fungi 11, 112.

Hobbie, E.A., 2006. Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology 87, 563–569.

Hobbie, E.A., Högberg, P., 2012. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. N. Phytol. 196, 367–382.

Huang, J., Lei, C., Shen, Y., Tian, E., 2024. Two new species of pholiota (Agaricales, Strophariaceae) from the southwest of China. MycoKeys 109.

Jacob, M., Weland, N., Platner, C., Schaefer, M., Leuschner, C., Thomas, F.M., 2009. Nutrient release from decomposing leaf litter of temperate deciduous forest trees along a gradient of increasing tree species diversity. Soil Biol. Biochem. 41, 2122–2130.

Johansson, E.M., Fransson, P.M.A., Finlay, R.D., van Hees, P.A.W., 2009. Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO2. Soil Biol. Biochem. 41, 1111–1116.

Kennedy, P.G., Peay, K.G., 2007. Different soil moisture conditions change the outcome of the ectomycorrhizal symbiosis between rhizopogon species and pinus muricata. Plant Soil 291, 155–165.

Kohler, A., Kuo, A., Nagy, L.G., et al., 2015. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. Nat. Genet. 47, 410–415.

Köhler, J., Yang, N., Pena, R., Raghavan, V., Polle, A., Meier, I.C., 2018. Ectomycorrhizal fungal diversity increases phosphorus uptake efficiency of european beech. N. Phytol. 220, 1200–1210.

Lang, A.K., Jevon, F.V., Vietorisz, C.R., Ayres, M.P., Hatala Matthes, J., 2021. Fine roots and mycorrhizal fungi accelerate leaf litter decomposition in a Northern hardwood forest regardless of dominant tree mycorrhizal associations. N. Phytol. 230, 316–326.

Langenfeld-Heyser, R., Gao, J., Ducic, T., Ph, Tachd, Lu, C.F., Fritz, E., Gafur, A., Polle, A., 2007. *paxillus involutus* mycorrhiza attenuate NaCl-stress responses in the salt-sensitive hybrid poplar Populus×canescens. Mycorrhiza 17, 121–131.

Leake, J.R., Donnelly, D.P., Saunders, E.M., Boddy, L., Read, D.J., 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following ^{14}C pulse labeling of *pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. Tree Physiol. 21, 71–82.

Lee, J.W., Park, M.S., Park, J.-H., Cho, Y., Kim, C., Kim, C.S., Jo, J.W., Lim, Y.W., 2020. Taxonomic study of the genus *pholiota* (Strophariaceae, Basidiomycota) in korea. Mycobiology 48, 476–483.

Lenth, R.V., 2024. emmeans Estim. Marg. Means aka LeastSq. Means (Version 1. 10. 0) [R. Package. ⟨https://CRAN.R-project.org/package=emmeans⟩.

Lindahl, B.D., Kyaschenko, J., Varenius, K., Clemmensen, K.E., Dahlberg, A., Karltun, E., Stendahl, J., 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. Ecol. Lett. 24, 1341–1351.

Lindahl, B., Stenlid, J., Finlay, R., 2001. Effects of resource availability on mycelial interactions and 32P transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. FEMS Microbiol. Ecol. 38, 43–52.

Lindahl, B., Stenlid, J., Olsson, S., Finlay, R., 1999. Translocation of 32P between interacting mycelia of a wood-decomposing fungus and ectomycorrhizal fungi in microcosm systems. N. Phytol. 144, 183–193.

Lindahl, B.D., Tunlid, A., 2015. Ectomycorrhizal fungi - potential organic matter decomposers, yet not saprotrophs. N. Phytol. 205, 1443–1447.

Liu, S.-L., Wang, X.-W., Li, G.-J., et al., 2024. Fungal diversity notes 1717–1817: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Divers. 124, 1–216.

Lucic, E., Fourrey, C., Kohler, A., Martin, F., Chalot, M., Brun-Jacob, A., 2008. A gene repertoire for nitrogen transporters in *laccaria bicolor*. N. Phytol. 180, 343–364.

Mangiafico, S.S., 2025. rcompanion: Functions to Support Extension Education Program Evaluation. Rutgers Cooperative Extension, New Brunswick, New Jersey version 2.5.0. https://CRAN.R-project.org/package=rcompanion.

Marschner, H., 2011. Marschner's mineral nutrition of higher plants. Academic Press.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51, 659–668.

Mayer, M., Matthews, B., Sandén, H., Katzensteiner, K., Hagedorn, F., Gorfer, M., Berger, H., Berger, T.W., Godbold, D.L., Rewald, B., 2023. Soil fertility determines whether ectomycorrhizal fungi accelerate or decelerate decomposition in a temperate forest. N. Phytol. 239, 325–339.

McBride, S.G., Choudoir, M., Fierer, N., Strickland, M.S., 2020. Volatile organic compounds from leaf litter decomposition alter soil microbial communities and carbon dynamics. Ecology 101, e03130.

Miller Jr., O.K., Volk,Thomas, J., Bessette, A.E., 1996. A new genus, leucopholiota, in the tricholomataceae (Agaricales) to accommodate an unusual taxon. Mycologia 88, 137–139.

Mu, X., Chen, Y., 2021. The physiological response of photosynthesis to nitrogen deficiency. Plant Physiol. Biochem. 158, 76–82.

Müller, A., Volmer, K., Mishra-Knyrim, M., Polle, A., 2013. Growing poplars for research with and without mycorrhizas. Front. Plant Sci. 4, 332.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.

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

Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. N. Phytol. 182, 31–48.

Näsholm, T., Persson, J., 2001. Plant acquisition of organic nitrogen in boreal forests. Physiol. Plant. 111, 419–426.

Nehls, U., Göhringer, F., Wittulsky, S., Dietz, S., 2010. Fungal carbohydrate support in the ectomycorrhizal symbiosis: a review. Plant Biol. 12, 292–301.

Nguyen, D., Pena, R., Polle, A., 2017. Impact of ectomycorrhizal community composition and soil treatment on inorganic nitrogen nutrition and performance of beech (*fagus sylvatica* L.) provenances. Trees 31.

Nicolás, C., Martin-Bertelsen, T., Floudas, D., Bentzer, J., Smits, M., Johansson, T., Troein, C., Persson, P., Tunlid, A., 2019. The soil organic matter decomposition mechanisms in ectomycorrhizal fungi are tuned for liberating soil organic nitrogen. ISME J. 13, 977–988.

Ogle, D.H., Doll, J.C., Wheeler, P., Dinno, A., 2024. FSA Fish. Stock Anal. (Version 0. 9. 5) [R. Package]. ⟨https://CRAN.R-project.org/package=FSA⟩.

Overholts, L.O., 1927. A monograph of the genus *pholiota* in the United States. Ann. Mo. Bot. Gard. 14, 87–211.

Pena, R., 2016. Nitrogen acquisition in ectomycorrhizal symbiosis. Molecular Mycorrhizal Symbiosis. John Wiley & Sons, Ltd, pp. 179–196.

Pena, R., Awad, A., Nawaz, A., Shang, Y., Wubet, T., Tibbett, M., 2025. Unravelling the facilitation-competition continuum among ectomycorrhizal and saprotrophic fungi. Soil Biol. Biochem. 208, 109865.

Pena, R., Bluhm, S.L., Ammerschubert, S., Agüi-Gonzalez, P., Rizzoli, S.O., Scheu, S., Polle, A., 2023. Mycorrhizal C/N ratio determines plant-derived carbon and nitrogen allocation to symbiosis. Commun. Biol. 6, 1230.

Pena, R., Polle, A., 2014. Attributing functions to ectomycorrhizal fungal identities in assemblages for nitrogen acquisition under stress. ISME J. 8, 321–330.

Pena, R., Simon, J., Rennenberg, H., Polle, A., 2013. *a*. Ectomycorrhiza *a*ffect *a*rchitecture *a*nd nitrogen partitioning of beech (*fagus sylvatica* L.) seedlings under shade *a*nd drought. Environ. Exp. Bot. 87, 207–217.

Pena, R., Tejedor, J., Zeller, B., Dannenmann, M., Polle, A., 2013b. Interspecific temporal and spatial differences in the acquisition of litter-derived nitrogen by ectomycorrhizal fungal assemblages. N. Phytol. 199, 520–528.

Pena, R., Tibbett, M., 2024. Mycorrhizal symbiosis and the nitrogen nutrition of forest trees. Appl. Microbiol. Biotechnol. 108, 461.

Pena, R., Tibbett, M. (Eds.), 2025. Physiological measurements and root morphology of poplar trees cultivated with different fungal guilds. NERC EDS Environmental Information Data Centre. https://doi.org/10.5285/82721bfc-d707-4255-bc5b-4d5882f9881b.

Pereira, E., Coelho, V., Tavares, R.M., Lino-Neto, T., Baptista, P., 2012. Effect of competitive interactions between ectomycorrhizal and saprotrophic fungi on *castanea sativa* performance. Mycorrhiza 22, 41–49.

Phillips, R.P., Brzostek, E., Midgley, M.G., 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. N. Phytol. 199, 41–51.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2023. nlme Linear Nonlinear Mixed Eff. ⟨https://CRAN.R-project.org/package=nlme⟩ Models (Version 3.1-162) [R package].

Read, D.J., 1991. Mycorrhizas in ecosystems. Experientia 47, 376–391.

Schreider, K., Boy, J., Sauheitl, L., Figueiredo, A.F., Andrino, A., Guggenberger, G., 2022. Designing a robust and versatile system to investigate nutrient exchange in, and partitioning by, mycorrhiza (*populus* x *canesces* x *paxillus involutus*) under axenic or greenhouse conditions. Front. Fungal Biol. 3.

Schröter, K., Wemheuer, B., Pena, R., Schöning, I., Ehbrecht, M., Schall, P., Ammer, C., Daniel, R., Polle, A., 2018. Assembly processes of trophic guilds in the root mycobiome of temperate forests. Mol. Ecol. https://doi.org/10.1111/mec.14887.

Setälä, H., McLean, M.A., 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139, 98–107.

Shah, F., Nicolás, C., Bentzer, J., et al., 2016. Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. N. Phytol. 209, 1705–1719.

Shaw, T.M., Dighton, J., Sanders, F.E., 1995. Interactions between ectomycorrhizal and saprotrophic fungi on agar and in association with seedlings of lodgepole pine (*pinus contorta*). Mycol. Res. 99, 159–165.

Shi, H., Lipka, U., Polle, A., 2024. Different ectomycorrhizal fungal species impact poplar growth but not phosphorus utilization under low p supply. Tree Physiol. 44, tpae074.

Smith, S.E., Read, D.J., 2010. Mycorrhizal symbiosis. Academic Press.

Šnajdr, J., Cajthaml, T., Valášková, V., Merhautová, V., Petránková, M., Spetz, P., Leppänen, K., Baldrian, P., 2011. Transformation of *quercus petraea* litter: successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. FEMS Microbiol. Ecol. 75, 291–303.

Soltangheisi, A., Hales-Henao, A., Pena, R., Tibbett, M., 2024. Species-specific effects of mycorrhizal symbiosis on *populus trichocarpa* after a lethal dose of copper. Ecotoxicol. Environ. Saf. 272, 116112.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R., Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. Soil Biol. Biochem. 57, 282–291.

Tibbett, M., Sanders, F.E., 2002. Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. Ann. Bot. 89, 783–789.

Urban, L., Aarrouf, J., Bidel, L.P.R., 2017. Assessing the effects of water deficit on photosynthesis using parameters derived from measurements of leaf gas exchange and of chlorophyll a fluorescence. Front. Plant Sci. 8, 2068. https://doi.org/10.3389/fpls.2017.02068.

Veen, G.F. (Ciska), Freschet, G.T., Ordonez, A., Wardle, D.A., 2015. Litter quality and environmental controls of home-field advantage effects on litter decomposition. Oikos 124, 187–195.

Verlinden, M.S., Ven, A., Verbruggen, E., Janssens, I.A., Wallander, H., Vicca, S., 2018. Favorable effect of mycorrhizae on biomass production efficiency exceeds their carbon cost in a fertilization experiment. Ecology 99, 2525–2534.

Warren, C., 2006. Estimating the internal conductance to CO2 movement. Funct. Plant Biol. 33, 431–442. https://doi.org/10.1071/FP05298.

Whalen, E.D., Lounsbury, N., Geyer, K., et al., 2021. Root control of fungal communities and soil carbon stocks in a temperate forest. Soil Biol. Biochem. 161, 108390.

Wickham, H., 2016. ggplot2: elegant graphics for. Data Analysis. Springer-Verlag New York. ⟨https://ggplot2.tidyverse.org⟩.

Wutkowska, M., Ehrich, D., Mundra, S., Vader, A., Eidesen, P.B., 2021. Can root-associated fungi mediate the impact of abiotic conditions on the growth of a high Arctic herb? Soil Biol. Biochem. 159, 108284.

Zadworny, M., Werner, A., Idzikowska, K., 2004. Behaviour of the hyphae of *laccaria laccata* in the presence of trichoderma harzianum in vitro. Mycorrhiza 14, 401–405.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecol. Monogr. 85, 133–155.

Zeller, B., Colin-Belgrand, M., Dambrine, E., Martin, F., Bottner, P., 2000. Decomposition of ^{15}N-labelled beech litter and fate of nitrogen derived from litter in a beech forest. Oecologia 123, 550–559.

Zhang, Z., Phillips, R.P., Zhao, W., Yuan, Y., Liu, Q., Yin, H., 2019. Mycelia-derived c contributes more to nitrogen cycling than root-derived c in ectomycorrhizal alpine forests. Funct. Ecol. 33, 346–359.