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Greater fine root biomass and endomycorrhizal colonization in the topsoil of a temperate forest under throughfall exclusion, with no detectable legacy effect

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Abstract

Background and Aims:

Temperate forests are projected to experience longer and more severe soil water deficits. Fine roots and their associated mycorrhizal fungi play a key role in soil resource acquisition, yet their plasticity and the persistence of their responses to water limitation remain poorly understood. We assessed the short-term and legacy effects of repeated growing-season soil water deficits on fine root depth distribution, mass density and fungal endophyte colonization in sugar maple (*Acer saccharum* Marsh.)-dominated forests.

Methods

At each of two sites, ~ 75% of throughfall was excluded from one plot during four consecutive growing seasons, while a second plot served as a control. Soil cores were collected in two subplots per plot during the final year of exclusion and the following year to measure fine root mass density and endomycorrhizal colonization at 0–10, 10–20 and 20–30 cm.

Results

During the final year of exclusion, fine root mass density at 0–10 cm was 80% higher in exclusion than in control plots (4.02 vs. 2.23 mg cm^{-3}), and endomycorrhizal colonization probability was fivefold higher (24.8% vs. 5.0%), whereas no treatment difference was detected at 10–30 cm. In the post-treatment year, neither variable differed between treatments.

Conclusion

These findings highlight the short-term plasticity of fine root mass density and endomycorrhizal colonization in sugar maple forests under growing-season water deficits, with no detectable legacy effect in the following growing season. Future studies should assess whether this topsoil response benefits tree water uptake during intermittent versus prolonged droughts.

1 Introduction

Climate change is expected to increase drought stress in temperate forests by altering precipitation regimes and increasing atmospheric water demand (Fusco et al. 2024; Zhao and Dai 2022). Although total annual precipitation is projected to increase in parts of eastern North America, greater evaporative demand, greater concentration of precipitation into extreme events and longer periods between precipitation events may increase the duration and severity of soil water deficits during the growing season (Cholet et al. 2022; Senecal et al. 2026). Drought may affect forest functioning without necessarily causing widespread tree mortality, by altering carbon assimilation, growth, water use and resource allocation, although these responses remain poorly understood (Nolet and Kneeshaw 2018; Wang et al. 2026). This is particularly the case in humid temperate forests, where evidence of drought impacts remains scarce because these forests have rarely experienced repeated and severe water deficits in recent decades (D'Orangeville et al. in press). Understanding these effects requires greater attention to

belowground processes, which play a central role in water and nutrient acquisition (Phillips et al. 2016). Belowground responses to water deficits remain less studied than aboveground responses (Brunner et al. 2015) and may persist beyond the period of water limitation (Müller and Bahn 2022). Examining how roots and their associated fungal communities respond to soil water deficits is therefore important for assessing forest response to and recovery from drought.

In eastern North America, sugar maple (*Acer saccharum* Marsh.) forests have experienced a progressive decline across the species' northern distribution range. While the specific causes remain uncertain, interactions between climatic stressors and nutrient imbalances are thought to contribute to this decline (Boakye et al. 2023). Soil acidification from atmospheric acid deposition can deplete nutrient cations, such as calcium (Ca) and magnesium (Mg), increasing tree sensitivity to secondary pathogens and aluminum (Al) toxicity (Côté and Ouimet 1996). Drought may further exacerbate these stresses by slowing litter decomposition (Parsons et al. 2025a), reducing soil nutrient supply (Parsons et al. 2025b) and uncoupling foliar nutrient concentrations from soil nutrient availability (Masson et al. 2024; Parsons et al. 2025b). Plant responses to drought range from drought avoidance, which limits water loss and maintains plant water status, to drought tolerance, which allows physiological functioning to continue despite declining water availability (Aubin et al. 2016). Sugar maple is generally considered a drought-avoiding species, reducing stomatal conductance during water stress to limit transpiration and maintain relatively stable water potential (Yi et al. 2017). However, how this drought-avoidance strategy relates to belowground resource acquisition remains unclear.

Fine roots and their associated fungi regulate belowground resource acquisition (Freschet et al. 2021) and can influence tree responses to drought (Brunner et al. 2015). Fine root mass density and depth distribution influence the capacity of root communities to exploit available soil water, with greater root abundance at depth potentially providing access to more stable water sources during drought as upper soil layers dry (Brinkmann et al. 2019; Neumann and Cardon 2012). Mycorrhizal associations can further enhance nutrient acquisition by extending the volume of soil explored beyond the root surface and potentially mitigating the effects of soil water deficits (Rapparini and Peñuelas 2014). Sugar maple primarily forms arbuscular mycorrhizal (AM) associations (Brundrett and Tedersoo 2020; Enea et al. 2025). Recent phylogenetic evidence has refined our understanding of this by distinguishing traditional AM fungi (subphylum *Glomeromycotina*) from fine root endophytes (FRE) belonging to subphylum *Mucoromycotina* (Orchard et al. 2017). These two groups may be functionally complementary: in liverworts, only *Mucoromycotina* symbionts provided substantial access to organic nitrogen (N), whereas both groups increased phosphorus (P) uptake, with *Glomeromycotina* often more effective (Field et al. 2019). Fungal access to P may be important when soil water deficits restrict P diffusion to roots (Gessler et al. 2017). Sugar maple roots can also host dark septate endophytes (DSE), whose effects on host plants range from beneficial, including improved nutrient uptake and abiotic stress tolerance, to neutral or detrimental (Enea et al. 2025; Mandyam and Jumpponen 2005). Overall, fine roots and their fungal associations may play key roles in ecosystem functioning during drought events.

The persistence of belowground responses to water limitation is relevant as droughts become more frequent, because incomplete recovery from one event may influence responses to subsequent water deficits. Root production, mortality, resource allocation, and plant–microbe interactions can be altered during drought and may remain altered during subsequent growing seasons (Müller and Bahn 2022). The magnitude and duration of these legacy effects depend partly on drought severity and on environmental conditions during recovery, including the availability of soil water following drought termination. Some ecosystems recover within a single growing season, whereas others exhibit changes that persist for several years (Vilonen et al. 2022; Yao et al. 2023). Recovery

patterns can be non-linear, for example when prolonged drought alters the coordination between above- and belowground traits (Ingrisch et al. 2023; Rowland et al. 2023).

The objective of this study was to assess the short-term and legacy effects of repeated growing-season soil water deficits on fine root depth distribution, mass density and endomycorrhizal colonization in sugar maple-dominated forests. We hypothesized that repeated soil water deficits would alter fine root distribution and endomycorrhizal colonization, with responses varying with soil depth and persisting into the next growing season. Given the drought-avoidance strategy of sugar maple, and because some temperate tree species shift water uptake to deeper soil layers when the topsoil dries (Brinkmann et al. 2019), we predicted greater fine root mass density and endomycorrhizal colonization in deeper soil layers, where water availability may be more stable. Conversely, we predicted lower endomycorrhizal colonization in shallow soil layers, where soil moisture is expected to decline more strongly in response to a water deficit. Finally, we predicted that these responses would persist into the year following the recovery of soil water availability, if soil moisture deficits were severe enough (Müller and Bahn 2022).

2 Materials and methods

2.1 Study area

The study took place in the temperate forest biome, within the private forest reserve of Kenauk Nature in southwestern Quebec (Canada; 45°45'N, 74°56'W; Online Resource 1, Fig. S1). Forest communities in the mesic sites of this region are dominated by sugar maple (*Acer saccharum* Marsh.), American basswood (*Tilia americana* L.), white ash (*Fraxinus americana* L.) and ironwood (*Ostrya virginiana* (Mill.) K. Koch). According to the Köppen-Geiger classification, the climate is humid continental with warm summers (Dfb; Beck et al. 2023). Based on climate normals for 1981–2010 at the two closest weather stations (< 6 km from the study sites), mean annual air temperature ranged from 4.8 to 5.3°C and mean annual precipitation from 986 to 1204 mm, with snowfall accounting for ~ 20% of precipitation (ECCC 2019). The soils are derived from glacial till containing gneiss, quartzite and granite and have a moder humus form. They are classified as Dystric Brunisols in the Canadian system (Soil Classification Working Group 1998) and as Typic Dystrudepts in the USDA system (Soil Survey Staff 2022). The study was conducted during the summers of 2024 and 2025 (May–July). In 2024, mean air temperature during these months was higher than the long-term average (17.2 vs. 15.8°C), and total precipitation was slightly higher (302 vs. 283 mm). In 2025, total precipitation was 17% lower than the long-term average (234 mm), indicating drier conditions during the early summer period. Air temperature data were not available for that period in 2025, preventing a comparison with the historical temperature average for that year.

2.2 Experimental design

We selected two sites (sites 5 and 7 of the throughfall exclusion experiment described by Parsons et al. 2025a), each comprising two 20 × 20 m plots. Within each site, one plot underwent a throughfall exclusion treatment, while the other served as a control. The sites were about 420 m apart. The plots were located on relatively flat ground in mid-slope positions, at an elevation of 256–271 m, and their mineral soil (0–15 cm) was a sandy loam (54–63% sand, 3–4% clay) with a pH (H_2O) of 4.35–5.18 (Online Resource 1, Table S1). The exclusion system consisted of 42 polyethylene tarpaulins suspended from ropes in a grid-like pattern 1 to 4 m above the ground to prevent throughfall from reaching the soil. Tarpaulins were cut around any interfering tree trunks or stems. The accumulated water following rainfall events was periodically emptied using bilge pumps. The tarpaulins were

estimated to intercept ~ 75% of throughfall. Throughfall exclusion was carried out from July to mid-September in 2021, and from late May to mid-September between 2022 and 2024. Each year, tarpaulins were removed in mid-September, allowing snow accumulation during the winter and the associated period of soil saturation during spring snowmelt.

Soil volumetric water content was measured across each plot to characterize soil water deficits induced by the throughfall exclusion treatment. Measurements were taken approximately every three weeks using a handheld time-domain reflectometry (TDR) moisture meter with a probe length of 12 cm (HS2 HydroSense II, Campbell Scientific Inc., Logan, UT, USA). Measurements were distributed in a grid-like pattern, with 42 measurement points per plot and three replicate measurements at each point. Relative extractable water (REW), which quantifies plant-available water, was calculated as (Vicca et al. 2012):

$$REW = \frac{\theta - \theta_{WP}}{\theta_{FC} - \theta_{WP}}$$

1

where θ corresponds to volumetric water content ($m^3\ m^{-3}$) measured by TDR in the upper 12 cm of soil, θ_{WP} is volumetric water content ($m^3\ m^{-3}$) at permanent wilting point (matric potential = − 1500 kPa) and θ_{FC} is volumetric water content ($m^3\ m^{-3}$) at field capacity (matric potential = − 33 kPa). Volumetric water content at field capacity and at permanent wilting point were calculated with the van Genuchten (1980) equation, using the residual and saturated water contents (θ_r, θ_s) and shape parameters (α, n) predicted by the Rosetta3 pedotransfer model (Zhang and Schaap 2017) from the sand, silt and clay contents measured in each plot in 2022 (θ_{FC} = 0.198–0.229 $m^3\ m^{-3}$; θ_{WP} = 0.073–0.078 $m^3\ m^{-3}$). Setting field capacity at − 33 kPa is consistent with the field capacity matric potential predicted from these parameters (− 29 to − 43 kPa; Assouline and Or 2014). REW facilitates comparisons across sites by accounting for differences in soil texture. Because field capacity and wilting point were estimated rather than measured, individual REW values can fall outside the 0–1 range. As a reference threshold, water vapor fluxes and gross primary production of European forests have been reported to decline when REW falls below about 0.4, owing to stomatal closure (Granier et al. 2007).

2.3 Fine roots and soil sampling

In each plot, fine roots were sampled in two subplots located in proximity to a mature sugar maple with a diameter at breast height (DBH) greater than 23 cm. Subplots were located at least 2 m from the boundaries of each plot to minimize edge effects. In 2024 (final year of exclusion), samples were collected 70 days after the exclusion system was installed (30–31 July 2024) to obtain a sufficient treatment effect and to coincide with a peak in fine root production (Burke and Raynal 1994). In 2025 (the year after treatment ended), sampling was conducted at a similar time of year (21–22 July 2025). Trees (DBH > 9 cm) and saplings (1 cm < DBH ≤ 9 cm) were inventoried in 2024 at the plot level (20 × 20 m) and at the subplot level, i.e. within a 4-m radius of each sampling point, to characterize species composition and the basal area of live stems.

To determine the root depth distribution and the effect of the treatment on community-level fine root mass density, undisturbed soil cores 30 cm deep were taken from microsites devoid of large rocks or roots using a cylindrical split auger 5.1 cm in diameter (AMS Inc., American Falls, ID, USA). Each soil core, taken after removal of the forest floor, was divided into three subsamples representing mineral soil depths of 0–10, 10–20, and 20–30 cm. This sampling depth covered the soil layers where sugar maple fine roots are most concentrated, with median sugar

maple fine root depths of about 12 cm in northern hardwood forests (Yanai et al. 2008). A second soil core was taken from each subplot to determine soil bulk density, which can vary with depth and influence resistance to root penetration.

Soil samples were stored at 4°C and weighed fresh no more than two weeks after sampling. The roots present in the samples were carefully separated from the soil particles using forceps. These were then rinsed by hand on a 0.5 mm sieve and divided using digital calipers into two diameter classes: 2–5 mm (intermediate) and ≤ 2 mm (fine). The latter were considered fine roots for the purposes of this study (Yanai et al. 2008). This method was employed to minimize potential losses of fine roots during sample cleaning (Fahey et al. 2017). Live and dead roots were distinguished using the visual indicators proposed by Hendrick and Pregitzer (1993), and dead roots were discarded. The cleaned roots were oven-dried at 60°C before weighing. The dry soil mass of each fine root sample was estimated by subtracting its water mass, calculated from the gravimetric water content of the corresponding bulk-density core, from the wet mass of the unseparated sample, and fine root mass per unit mass of dry soil (g g^{-1}) was calculated by dividing fine root dry mass by this dry soil mass. Fine root mass density (mg cm^{-3}), i.e. fine root dry mass per unit soil volume (Jaeger et al. 2024), was then obtained by multiplying this value by the soil bulk density of the corresponding depth (Billings et al. 2025; NRCS 2001):

$$\text{root mass density } \left(\text{mg cm}^{-3}\right) = \frac{\text{root dry mass } \left(\text{g}\right)}{\text{soil dry mass } \left(\text{g}\right)} \times \text{ soil bulk density } \left(\text{g cm}^{-3}\right) \times 1000 \left(\text{mg g}^{-1}\right)$$

2

This approach accounts for differences in soil density among depths, including the looser, organic-rich upper soil horizons, as well as spatial variability associated with forest heterogeneity and microtopography, which can influence root growth (Archer and Smith 1972; Tauc et al. 2020).

2.4 Fine root fungal endophyte colonization

A subsample of soil containing fine roots was withdrawn from each soil bulk density core at each depth to determine the fungal endophyte colonization rate of fine roots. The fine roots were preserved in moist soil under vacuum conditions to prevent degradation due to desiccation and cellular respiration and were stored at 4°C for a maximum of four months. The gridline intersect method (Giovannetti and Mosse 1980) was then used to estimate the colonization rate. Although this method is prone to overestimation and does not allow some colonizing fungal taxa to be precisely identified, it remains a widely used method (Kokkoris et al. 2019). To prepare the fine roots for analysis, they were washed using the previously described technique and stained using the Phillips and Hayman (1970) method. The process involved clarification of samples in a boiling alkaline solution (KOH), subsequent oxidation and depolymerization in hydrogen peroxide (H_2O_2) to remove tannins of woody plant roots, acidification with hydrochloric acid (HCl) and histological staining in a boiling solution of trypan blue (Direct Blue 14). Our study employed a modified approach developed by Dalpé and Séguin (2013), which uses microwave-assisted heat treatment for clearing and staining.

After clearing and staining the roots, 60–90 segments, each measuring 1–2 cm, were randomly selected from each sample and arranged in a Petri dish marked with a 1 × 1 cm grid. Colonization rate estimation was carried out by visualizing the intersections of grid lines and roots, with each intersection being recorded where the presence of fungal endophytes was detected (Sun and Tang 2012). A total of 100 grid line and root intersection points were counted for each sample, with some samples requiring the roots to be remixed at random to reach this number.

Because vesicles and arbuscules were not frequently observed, hyphae were used as the main indicator of colonization. However, aseptate hyphae were attributed to arbuscular mycorrhizae only when connected to vesicles, arbuscules or external chlamydospores. Dark septate endophyte (DSE) hyphae were distinguished from arbuscular mycorrhizal (AM) hyphae by septate internal cross-walls when possible and by the presence of nearby microsclerotia. Fine root endophytes (FRE) could not be reliably differentiated from AM and were therefore included in AM counts, so that our overall assessment represents endomycorrhizal (AM + FRE) colonization. Endomycorrhizal and DSE colonization rates were calculated separately as the number of colonized intersections divided by the total number of root-gridline intersections.

2.5 Statistical analysis

Fine root mass density was modeled with a generalized linear mixed model (GLMM) using a Gamma family (log link) with fixed effects of soil depth (categorical: 0–10 cm, 10–20 cm, 20–30 cm), treatment (categorical: exclusion, control), their interaction and site. A random intercept for subplot was included to account for the non-independence of measurements across soil depths within subplots. Endomycorrhizal colonization rate (expressed as the proportion of 100 root-gridline intersections showing colonization) was modeled using a beta-binomial family (logit link; 100 trials per sample) to account for overdispersion in the proportional data, using the same fixed and random effects structure. To test whether treatment effects persisted into the post-treatment year, the paired difference between years (2025 minus 2024) in fine root mass density and in endomycorrhizal colonization rate was calculated for each subplot and depth and modeled with a Gaussian family (identity link) and the same fixed and random effects. In these models, the exclusion–control contrast at a given depth estimates the change in the treatment effect between years. Post hoc pairwise comparisons of estimated marginal means were performed using the emmeans package (v1.8.5; Lenth 2023). Tukey adjustments were used for pairwise comparisons of depths within each treatment and a multivariate t-distribution was used for contrasts of treatments within each depth. Effects were considered significant at $p < 0.05$. To report estimated marginal means on the observation scale, we applied a bias-adjusted back-transformation that incorporates the random effect variance, to approximate population-averaged means for non-linear link functions. DSE colonization was not modeled because it was low in both years.

For the yearly models, fixed effect estimates and contrasts were exponentiated to obtain response ratios (RR, log link) or odds ratios (OR, logit link), which represent the response in the exclusion treatment, or at a given depth, relative to the reference level. For example, a response ratio of 1.20 indicates a 20% higher fine root mass density in exclusion than in control plots, and an odds ratio of 2.00 indicates that the odds of a root–gridline intersection being colonized were twice as high. For the difference models, estimates are expressed on the original scale, as differences in mg cm^{-3} or in colonization proportion, so that a negative exclusion–control contrast indicates a larger decrease between years in previously excluded than in control subplots. All estimates are reported with their 95% confidence intervals.

We quantified the models' explanatory power using marginal and conditional R^2 (Nakagawa and Schielzeth 2013; Nakagawa et al. 2017), and model assumptions (residual uniformity, dispersion, outliers and homoskedasticity) were assessed with simulated residuals using the DHARMa package (v0.4.7; Hartig 2024). Residual autocorrelation among depths within cores was tested with a Durbin–Watson test (performance package v0.16.0; Lüdecke et al. 2021). Mixed effects models were fitted with the glmmTMB package (v1.1.5; Brooks et al. 2017), all statistical analyses were conducted in R (v4.2.3; R Core Team 2023), and figures were produced with the ggplot2 package (v3.4.3; Wickham 2016).

3 Results

3.1 Forest structure and species composition

Total (trees and saplings) basal area differed between control and exclusion plots (20 × 20 m), with the direction of the difference varying between sites. At site 5, total basal area was lower in the exclusion than in the control plot (35.6 vs. 42.3 m^2 ha^{-1}) whereas the opposite pattern was observed at site 7 (37.1 vs. 31.4 m^2 ha^{-1}). A similar pattern was observed for tree (DBH > 9 cm) basal area, which corresponded to 34.0 and 40.6 m^2 ha^{-1} in exclusion and control plots, respectively, at site 5 and 35.3 and 29.3 m^2 ha^{-1} at site 7. At the plot level, sugar maple dominated, representing between 76 and 93% of total basal area and 78 and 94% of tree basal area. The sapling layer (DBH ≤ 9 cm) was dominated by ironwood at site 7 and shared between sugar maple and ironwood at site 5.

At the subplot level (4-m radius around fine root sampling position), total basal area ranged from 23.1 to 63.4 m^2 ha^{-1}, with sugar maple accounting for 83.1 to 99.8% of total basal area. At site 7, sugar maple tree basal area was higher on average in the exclusion subplots than in the control subplots (22.0 and 61.4 vs. 20.1 and 31.6 m^2 ha^{-1}), mainly because exclusion subplots contained more sugar maple trees (two and six vs. one and two). At the fine root sampling points, the litter (L) and fermentation (F) layers of the forest floor were on average thicker in exclusion than in control plots at both sites (L: 2.7–5.6 vs. 1.2–1.8 cm; F: 3.7–3.9 vs. 1.8–2.6 cm; Table S1).

3.2 Throughfall exclusion effectively reduced soil water availability

In 2024, the throughfall exclusion treatment created drier soil conditions, with control plots having a consistently higher REW than the exclusion plots throughout the treatment period (Fig. 1a). At the last soil moisture measurements before fine root sampling (15 July at site 5 and 24 July at site 7, corresponding to 15 and 6 days before fine root sampling, respectively), median plot-level REW was lower in exclusion than in control plots at both site 5 (0.30 vs. 0.91) and site 7 (0.16 vs. 0.41). At both sites, median REW in the exclusion plots was below 0.4, a threshold associated with the onset of soil water limitation and stomatal closure. REW showed considerable within-plot variability (Fig. 1), which likely reflects small-scale differences in microtopography and greater soil moisture near the margins of the exclusion plots. In addition, occasional tarpaulin failures, although rapidly repaired, locally raised soil moisture and may have also increased variability and reduced the contrast in soil moisture between exclusion and control plots. Nevertheless, the exclusion treatment maintained drier soil conditions during the period preceding fine root sampling.

In 2025, the year after treatment ended, REW was generally comparable between previously excluded plots and control plots (Fig. 1b). Soil moisture measured before fine root sampling (9 July, 12–13 days before sampling) showed that median plot-level REW ranged from 0.85 to 1.34 across previously excluded and control plots, above the 0.4 threshold, indicating no substantial soil water limitation.

3.3 Greater fine root mass density in the upper soil layer under exclusion, but no persistent effect in the following year

In 2024, fine root mass density was higher in exclusion than in control plots in the upper soil layer (0–10 cm), but not in deeper soil layers (Fig. 2a). At 0–10 cm, fine root mass density under exclusion was 80% higher than under control conditions (RR = 1.80, p = 0.007; Table 1), and it was higher in each exclusion subplot than in either control subplot of the same site. No significant differences were observed between the exclusion and control plots at 10–

20 cm or 20–30 cm ($p \geq 0.90$). In 2025, the year after the exclusion treatment ended, there was no significant effect of the previous exclusion treatment on fine root mass density (Fig. 2b, Table 1). At 0–10 cm, the estimated marginal mean fine root mass density was 2.55 mg cm^{-3} in previously excluded plots in 2025, lower than in the same plots in 2024 (4.02 mg cm^{-3}) and close to 2024 control levels (2.23 mg cm^{-3}). Accordingly, the change between years at 0–10 cm differed between treatments, with fine root mass density decreasing by 1.36 mg cm^{-3} more in previously excluded than in control subplots ($p = 0.048$), whereas no difference in change between treatments was detected at 10–20 or 20–30 cm ($p \geq 0.90$; Table 1). The higher fine root mass density observed at 0–10 cm under exclusion in 2024 therefore converged toward control levels in the post-treatment year.

In 2024, fine root mass density was 1.8 to 3.0 times higher at 0–10 cm than at 10–20 and 20–30 cm under both exclusion and control conditions (RR = 1.79–3.02, $p < 0.01$; Table 1). Depth contrasts appeared stronger under exclusion (RR = 2.86–3.02) than under control conditions (RR = 1.79–1.89), although the 0–10 cm × exclusion interaction term was not significant ($p = 0.086$; Table S2). A similar vertical pattern was observed in 2025 after the exclusion ended, with fine root mass density remaining significantly greater in the 0–10 cm layer than in the deeper soil layers (RR = 1.57–2.37; Table 1). Soil bulk density increased with depth at both sites, averaging 0.67, 0.87, and 0.97 g cm^{-3} at 0–10, 10–20, and 20–30 cm, respectively, across both years, with this pattern consistent between exclusion and control plots (Fig. S2). Because fine root mass density was calculated per unit soil volume, its decline with depth was not driven by this increase in bulk density. Fine root mass density was higher at site 7 than at site 5 in 2024 ($p < 0.001$), but not significantly so in 2025 ($p = 0.063$; Table S2). Overall, models explained a substantial proportion of variation in fine root mass density, with marginal R^2 values of 0.81 in 2024, 0.70 in 2025 and 0.39 for the difference model. Residual autocorrelation among depths was detected only in the 2025 model (Durbin–Watson test, $p < 0.001$), which may have led to underestimated standard errors for this year.

Table 1

Contrasts of estimated marginal means of fine root mass density in 2024 (during throughfall exclusion), in 2025 (the year after exclusion ended) and for the change between years (2025 − 2024, paired by subplot and depth). Yearly models give response ratios (RR) and the difference model gives differences in mg cm^{-3}, a negative value indicating a larger decrease between years for the first level of the contrast. Depth contrasts are Tukey-adjusted and treatment contrasts are adjusted with a multivariate t-distribution; fixed-effect estimates are given in Table S2

Contrast	2024 RR [95% CI]	*p*	2025 RR [95% CI]	*p*	2025 − 2024 difference (mg cm^{-3}) [95% CI]	*p*
Control: 0–10 vs. 10–20 cm	1.79 [1.14, 2.82]	0.007	1.57 [1.14, 2.18]	0.003	−0.15 [− 1.48, 1.18]	0.962
Control: 0–10 vs. 20–30 cm	1.89 [1.20, 2.97]	0.003	1.95 [1.41, 2.69]	< 0.001	0.06 [− 1.28, 1.39]	0.995
Control: 10–20 vs. 20–30 cm	1.05 [0.67, 1.66]	0.959	1.24 [0.89, 1.72]	0.277	0.21 [− 1.13, 1.54]	0.930
Exclusion: 0–10 vs. 10–20 cm	2.86 [1.82, 4.50]	< 0.001	2.14 [1.55, 2.97]	< 0.001	−1.17 [− 2.50, 0.17]	0.101
Exclusion: 0–10 vs. 20–30 cm	3.02 [1.92, 4.75]	< 0.001	2.37 [1.72, 3.29]	< 0.001	−1.13 [− 2.46, 0.21]	0.117
Exclusion: 10–20 vs. 20–30 cm	1.06 [0.67, 1.66]	0.958	1.11 [0.80, 1.53]	0.738	0.04 [− 1.29, 1.37]	0.997
Exclusion vs. control, 0–10 cm	1.80 [1.14, 2.86]	0.007	1.24 [0.83, 1.84]	0.494	−1.36 [− 2.72, − 0.01]	0.048
Exclusion vs. control, 10–20 cm	1.13 [0.71, 1.79]	0.901	0.91 [0.61, 1.35]	0.902	−0.35 [− 1.71, 1.01]	0.902
Exclusion vs. control, 20–30 cm	1.13 [0.71, 1.79]	0.902	1.01 [0.68, 1.51]	0.998	−0.18 [− 1.54, 1.17]	0.984

3.4 Greater endomycorrhizal colonization in the upper soil layer under exclusion, but no persistent effect in the following year

In 2024, endomycorrhizal colonization was higher in exclusion than in control plots in the upper soil layer, but not in deeper soil layers (Fig. 3a). At 0–10 cm, endomycorrhizal colonization probability was fivefold higher in exclusion plots than under control conditions, with estimated marginal means of 24.8% and 5.0%, respectively (OR = 6.66, p = 0.009; Table 2), and colonization was higher in each exclusion subplot than in either control subplot of the same site. No significant differences in endomycorrhizal colonization were detected between exclusion and control plots at 10–20 or 20–30 cm. The response to exclusion appeared to vary between sites, with markedly higher colonization in exclusion plots at all soil depths at site 7, but only in the 0–10 cm layer at site 5 (Fig. 3a). This site-specific pattern may explain why the difference between exclusion and control plots at 10–20 cm was not significant (p = 0.119; Table 2).

In 2025, the year after the exclusion treatment ended, there was no significant effect of the previous exclusion treatment on endomycorrhizal colonization (Fig. 3b, Table 2). At 0–10 cm, the estimated marginal mean endomycorrhizal colonization was 3.8% in previously excluded plots in 2025, lower than in the same plots in 2024 (24.8%) and similar to the 2024 control level (5.0%). Accordingly, the change in colonization between years differed between treatments at 0–10 cm (− 0.22, p = 0.018) and 10–20 cm (− 0.19, p = 0.045), but not at 20–30 cm (p =

0.902), indicating that endomycorrhizal colonization in previously excluded plots converged toward control levels after the exclusion treatment ended (Table 2).

Across soil depths, endomycorrhizal colonization showed no consistent vertical pattern in either year. In 2024, endomycorrhizal colonization did not differ significantly among soil depths within either treatment, although estimated colonization in control plots was lower at 0–10 cm than at 10–20 cm (OR = 0.36, p = 0.060) and 20–30 cm (OR = 0.39, p = 0.104; Table 2). In 2025, colonization in previously excluded plots was lower at 0–10 cm (OR = 0.21, p < 0.001) and 10–20 cm (OR = 0.37, p = 0.016) than at 20–30 cm, whereas no depth difference was detected in control plots (Table 2). Models explained a substantial proportion of variation in endomycorrhizal colonization, with marginal R^2 values of 0.52 in 2024, 0.63 in 2025 and 0.42 for the difference model. Residual autocorrelation among depths was detected only in the 2024 model (Durbin–Watson test, p = 0.004), which may have led to underestimated standard errors for this year. No formal analysis was conducted for DSE colonization rate because it was generally low: DSE colonization was ≤ 1% in 16 of the 24 samples and < 5% in 22 of the 24 samples in 2024, and no DSE structures were observed in 12 of the 24 samples in 2024 and in 9 of the 24 samples in 2025 (Fig. S3).

Table 2
Contrasts of estimated marginal means of endomycorrhizal colonization in 2024 (during throughfall exclusion), in 2025 (the year after exclusion ended) and for the change between years (2025 − 2024, paired by subplot and depth). Yearly models give odds ratios (OR) and the difference model gives differences in colonization proportion, a negative value indicating a larger decrease between years for the first level of the contrast. Adjustments as in Table 1; fixed-effect estimates are given in Table S3

Contrast	**2024 OR [95% CI]**	***p***	**2025 OR [95% CI]**	***p***	**2025 − 2024 difference (proportion) [95% CI]**	***p***
Control: 0–10 vs. 10–20 cm	0.36 [0.12, 1.03]	0.060	0.63 [0.27, 1.48]	0.420	0.04 [− 0.13, 0.20]	0.857
Control: 0–10 vs. 20–30 cm	0.39 [0.13, 1.15]	0.104	0.60 [0.26, 1.41]	0.341	0.02 [− 0.15, 0.19]	0.957
Control: 10–20 vs. 20–30 cm	1.10 [0.47, 2.56]	0.960	0.95 [0.44, 2.06]	0.988	−0.02 [− 0.18, 0.15]	0.967
Exclusion: 0–10 vs. 10–20 cm	0.79 [0.41, 1.51]	0.664	0.55 [0.18, 1.70]	0.429	0.01 [− 0.15, 0.18]	0.983
Exclusion: 0–10 vs. 20–30 cm	1.18 [0.59, 2.36]	0.850	0.21 [0.08, 0.56]	< 0.001	−0.15 [− 0.31, 0.02]	0.094
Exclusion: 10–20 vs. 20–30 cm	1.49 [0.76, 2.95]	0.349	0.37 [0.16, 0.86]	0.016	−0.16 [− 0.33, 0.01]	0.062
Exclusion vs. control, 0–10 cm	6.66 [1.50, 29.67]	0.009	0.47 [0.14, 1.57]	0.348	−0.22 [− 0.40, − 0.03]	0.018
Exclusion vs. control, 10–20 cm	3.01 [0.81, 11.13]	0.119	0.54 [0.20, 1.47]	0.367	−0.19 [− 0.38, − 0.01]	0.045
Exclusion vs. control, 20–30 cm	2.22 [0.58, 8.44]	0.342	1.39 [0.59, 3.26]	0.727	−0.05 [− 0.23, 0.14]	0.902

4 Discussion

Our results indicate a plastic belowground response to growing-season water limitation, characterized by greater fine root mass density and endomycorrhizal colonization in the topsoil of sugar maple-dominated forests under

throughfall exclusion. These responses were temporary, with both fine root mass density and endomycorrhizal colonization converging toward control levels after the treatment ended, suggesting no detectable legacy effect into the next growing season.

4.1 Topsoil fine root foraging as a response to water limitation

Contrary to our prediction, growing-season water limitation was associated with greater fine root mass density in the topsoil rather than in deeper soil layers, where we had predicted more extensive fine root development under more stable and available water resources (Fig. 2a). This response indicates belowground plasticity in sugar maple-dominated forests, with greater investment in fine roots potentially helping trees maintain resource acquisition under reduced soil moisture availability (Weemstra et al. 2020). Sugar maple is generally characterized by a drought-avoidance strategy involving coordinated regulation of water loss through stomatal closure and water uptake. Greater fine root mass can contribute to this strategy by maintaining water uptake under water-limiting conditions (Brunner et al. 2015). Although our study sites were dominated by sugar maple, we cannot attribute the observed belowground response specifically to this species because root-level species identification was not possible. Rather than shifting root development toward deeper soil layers, the studied forests had a higher density of fine roots in the topsoil under exclusion, which may facilitate access to resources near the soil surface under water limitation.

The concentration of fine root development in the topsoil may reflect the greater availability of both water and nutrients in this soil layer. First, shallow root systems can rapidly capture small precipitation pulses that may wet the upper soil layers without substantially replenishing deeper soil water (Joseph et al. 2020; Natkhin et al. 2026). Second, the topsoil generally contains greater concentrations of macronutrients (N, P, K) than deeper soil layers, making it an important resource zone when drought reduces nutrient mobility and microbial activity (Gessler et al. 2017; Jobbágy and Jackson 2001; Lynch 2022). In sugar maple forests where cation depletion from atmospheric acid deposition contributes to nutritional stress, maintaining an extensive topsoil root system may also help sustain access to nutrient cations such as Ca and Mg (Long et al. 2009). This interpretation is relevant to our study sites, where Parsons et al. (2025b) reported that foliar Mg insufficiency in sugar maple increased under throughfall exclusion. Overall, greater fine root development in the topsoil may have allowed trees to maintain access to soil resources concentrated in the upper soil layer under simultaneous water and nutrient limitation.

Our results are consistent with previous experimental evidence of greater fine root biomass in sugar maple forests under reduced precipitation. Using a throughfall exclusion experiment in a sugar maple-dominated forest in West Virginia (USA), about 800 km south-west of our study sites, Raczka et al. (2023) also reported greater fine root biomass in the upper 15 cm of mineral soil under reduced precipitation. However, their study did not examine how this response varied with soil depth. Our results therefore extend these findings by showing that greater fine root mass density was detected only in the upper 10 cm of mineral soil. Similarly, young sugar maple trees exposed to contrasting growing-season water availability showed high plasticity of absorptive fine root biomass in the upper 5 cm of soil but low plasticity in rooting depth (Jaeger et al. 2024).

The topsoil fine root response contrasts with the shift of water uptake toward deeper soil layers reported during drought for other temperate tree species (*Fraxinus excelsior*, *Fagus sylvatica*, *Acer pseudoplatanus*; Brinkmann et al. 2019), although we did not measure where water was taken up. Our findings also contrast with the limited belowground plasticity reported for some species. For example, Raczka et al. (2023) found no change in belowground carbon investment in response to a throughfall exclusion in an oak-dominated forest (*Quercus*

velutina Lam., *Quercus rubra* L., *Quercus alba* L.), potentially reflecting a higher baseline investment in belowground carbon. The characteristically shallow rooting system of sugar maple (Biswell 1935; Jaeger et al. 2024) and concentration of fine roots near the soil surface in northern hardwood forests (Hendrick and Pregitzer 1996; Yanai et al. 2008) may partly explain why greater fine root mass density was detected only in the topsoil. Fine roots were also concentrated near the surface in our control plots, where fine root mass density was 1.6 to 2.0 times higher at 0–10 cm than at 10–20 and 20–30 cm in both years (Table 1). Together, these contrasting responses highlight variability in fine root responses to water limitation among temperate forests with different species compositions.

4.2 Enhanced endomycorrhizal colonization in the topsoil under water limitation

In line with our hypothesis, endomycorrhizal colonization was higher under throughfall exclusion, but contrary to our prediction, this difference was detected only in the upper soil layer, where colonization was fivefold greater in exclusion than in control plots, and not in deeper soil layers (Fig. 3a). Part of this difference reflected the low colonization of control roots at 0–10 cm, which was lower than at 10–30 cm, although not significantly (Table 2), so the restriction of this response to the topsoil should be interpreted with caution. The higher endomycorrhizal colonization coincided with greater fine root mass density at 0–10 cm, indicating that both fine root biomass and root–fungal associations responded to water limitation. However, whether these concurrent responses enhance water or nutrient acquisition cannot be determined from our measurements, although AM symbioses have been shown to mitigate the effects of water limitation across a wide range of host plants (Augé 2001; Jayne and Quigley 2014), and AM fungal hyphae can transport water to host plants (Kakouridis et al. 2022).

This response differs in part from that reported in another throughfall exclusion experiment in a sugar maple-dominated forest. Raczka et al. (2023) found no change in AM colonization under 50% throughfall exclusion in the first year, a marginal increase in the second year, and a 58% decrease in the third year, when 90% of throughfall was excluded during the whole growing season. The higher colonization observed after four years of ~ 75% exclusion in our study therefore suggests that the endomycorrhizal response to water limitation may depend on its severity and duration, and that higher colonization may not be maintained under more severe deficits.

In our study, DSE colonization remained low across both sampling years, with no DSE structures observed in 9 to 12 of the 24 samples each year. Moreover, DSE colonization showed no consistent response to throughfall exclusion, being higher under exclusion only at 10–30 cm at site 7 in 2024 (Fig. S3), despite evidence that DSE associations can contribute to plant tolerance to environmental stress in some species (Li et al. 2019; Mandyam and Jumpponen 2005). The low colonization rates and lack of consistent response to water limitation may reflect differences in host affinity or environmental conditions. As the functional role of DSE in sugar maple is poorly documented (Enea et al. 2025), the significance of their limited response to water limitation remains unclear.

4.3 Fine root responses to water limitation did not persist into the following growing season

Following the end of throughfall exclusion, fine root mass density and endomycorrhizal colonization converged toward levels comparable to those observed in control plots, and the treatment effects observed at 0–10 cm in 2024 decreased significantly between years (Figs. 2b, 3b; Tables 1, 2). Overall, we detected no persistent legacy of water limitation on fine root mass density or endomycorrhizal colonization in the growing season following the exclusion, when soil moisture before fine root sampling was not limiting. This rapid recovery contrasts with dendroclimatological evidence of multi-year reductions in basal area increment following climatic stress events,

including droughts (Moreau et al. 2020; Nolet and Kneeshaw 2018), suggesting that the persistence of drought effects may differ between belowground and aboveground processes. Similarly, at the global scale, drought legacy effects on ecosystem gross primary production were weaker than concurrent drought effects and were mainly detected in drier regions (Liu et al. 2026), consistent with the absence of a detectable legacy at our mesic sites. In contrast, in the same experiment and year, sugar maple foliage in previously excluded plots still showed a relative enrichment in K compared with N and P once soil moisture had recovered (Simoneau et al. 2026), suggesting that drought effects on foliar nutrient ratios can persist after fine root responses have disappeared.

The recovery of fine root mass density and endomycorrhizal colonization may reflect the rapid turnover of fine roots in forest ecosystems. In sugar maple-dominated northern hardwood forests, fine root turnover has been estimated at 0.5–1.2 yr^{-1} (Burke and Raynal 1994; Burton et al. 2000), meaning that a large part of the standing fine root biomass is renewed within one to two years. This high turnover likely enables fine root biomass to adjust rapidly to changing environmental conditions (Hou et al. 2024; Kubisch et al. 2016). For example, belowground carbon allocation and rhizosphere metabolic activity increased rapidly after a small rainfall event in a naturally dry mature Scots pine forest (Joseph et al. 2020). Similarly, after five years of experimental summer drought, fine root vitality of *Picea abies* resembled that of control trees 17 days after rewetting, whereas the recovery of *Fagus sylvatica* was delayed and not sustained over the following three months (Danzberger et al. 2023), highlighting species-specific recovery trajectories in temperate mixed forests. Thus, the rapid recovery observed in our study and reported in other temperate forests suggests that fine root responses to water limitation may be relatively transient when soil water availability is restored.

4.4 Limitations

These belowground responses should be interpreted in the context of the limitations inherent to large-scale throughfall exclusion experiments. The practical difficulty of installing and maintaining large exclusion structures necessarily limited the number of treatment plots (n = 2), increasing the potential for spatial pseudoreplication (Hurlbert 1984). Statistical inference therefore applies to the sampled plots, and generalization to other sugar maple forests rests on the consistency of the responses between the two sites. Because subplots were nested within a single treatment plot at each site, and no pre-treatment root measurements were available, differences between treatments may also partly reflect pre-existing variation in soil properties (Table S1), microtopography or forest structure, such as moisture differences between pits and mounds or differences in sugar maple basal area among subplots, rather than treatment effects alone (Tauc et al. 2020; Valtera and Schaetzl 2017). Soil moisture was measured only in the upper 12 cm, so the extent of water limitation in deeper soil layers was not documented. The absence of deep perimeter trenches, which were not feasible because of rocky soils, may have allowed lateral subsurface water movement or roots extending beyond the exclusion area to provide additional water to treatment plots, potentially reducing the severity of water limitation, especially at greater soil depths (Asbjornsen et al. 2018). In addition, fine root mass density represents standing root biomass and therefore cannot distinguish whether differences resulted from changes in root production, root mortality, or both. Fine roots were also not sampled in the forest floor, which was thicker in exclusion plots (Table S1), possibly because of slower litter decay under exclusion (Parsons et al. 2025a), so a redistribution of fine roots between the forest floor and the upper mineral soil cannot be excluded. Finally, although the gridline intersect method provides a practical and widely used measure of fungal colonization, it is based on visual identification of stained fungal structures and does not allow reliable identification of fungal groups such as *Glomeromycotina* and *Mucoromycotina* in mixed samples (Hoysted et al. 2019; Orchard et al. 2017).

5 Conclusions

We found that sugar maple-dominated forests responded to growing-season water limitation primarily through an adjustment of their belowground system in the topsoil. Fine root mass density and endomycorrhizal colonization were greater in the upper 0–10 cm of soil under throughfall exclusion, whereas no significant differences were detected at greater depths. This pattern contrasts with our prediction that water limitation would promote greater root development in deeper soil layers, where water availability may be more stable, but is consistent with the greater topsoil fine root biomass reported in a similar experiment in a sugar maple-dominated forest. These responses were not maintained into the year following the end of throughfall exclusion, when soil moisture before fine root sampling was not limiting, indicating no detectable legacy effect and suggesting that the belowground system can rapidly converge toward control levels once water limitation ends. Overall, these findings highlight the plasticity of the fine root system of sugar maple forests under water deficits during the growing season. Because summer rainfall in humid temperate forests often rewets only the upper soil layers, such a topsoil response may facilitate water uptake following rainfall during intermittent water deficits but could constrain water acquisition during prolonged droughts if access to deeper soil water remains limited. Future studies combining greater spatial replication, measurements of root production and turnover and taxonomically resolved characterization of fungal communities will help clarify the mechanisms underlying these responses and their implications for the drought resilience of sugar maple forests.

Abbreviations

AM
arbuscular mycorrhizal
CI
confidence interval
DBH
diameter at breast height
DSE
dark septate endophytes
FRE
fine root endophytes
GLMM
generalized linear mixed model
OR
odds ratio
REW
relative extractable water
RR
response ratio
SD
standard deviation
TDR
time-domain reflectometry

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval and consent

Not applicable. The study did not involve human participants or animals.

Supplementary Information

Online Resource 1: map of the study area and plots (Fig. S1), location, topography, soil and stand characteristics of the plots (Table S1), fixed-effect estimates of the fine root mass density and endomycorrhizal colonization models (Tables S2, S3), and soil bulk density and dark septate endophyte colonization at 0–10, 10–20 and 20–30 cm (Figs. S2, S3).

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Author contributions

T. Monette contributed conceptualization, data curation, methodology, investigation, visualization, formal analysis and writing (original draft, review and editing). P. Arnault contributed data curation and investigation. A. Maheu contributed conceptualization, methodology, investigation, supervision, funding acquisition, and writing (review and editing). All authors read and approved the final manuscript.

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Data availability

The data and R code supporting the findings of this study are openly available in the Zenodo repository at https://doi.org/10.5281/zenodo.23672857.

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Figures

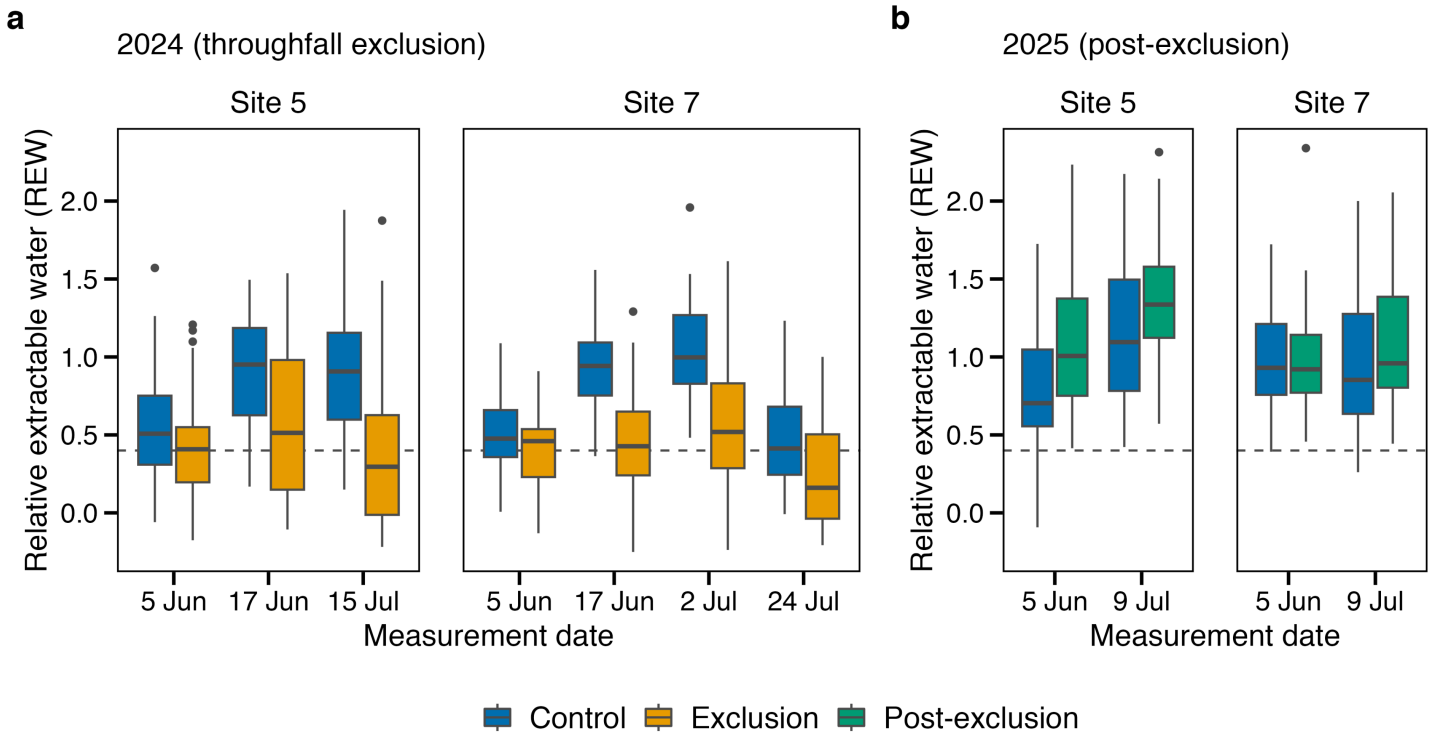


Figure 1

Relative extractable water (REW), calculated from TDR measurements in the upper 12 cm of soil, in control and exclusion plots at sites 5 and 7 (a) in 2024, during throughfall exclusion, and (b) in 2025, the year after exclusion ended (post-exclusion). Boxplots show the 42 measurement points of each plot on each date preceding fine root sampling; the dashed line indicates REW = 0.4

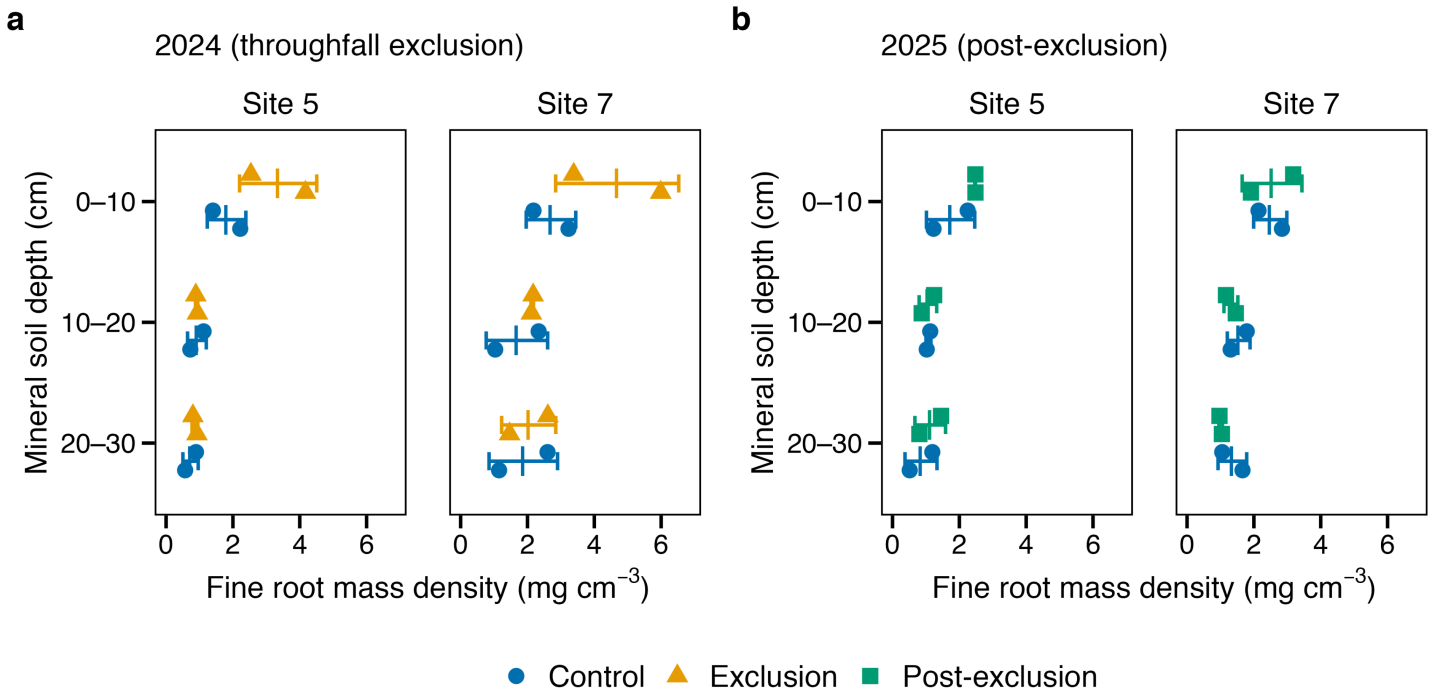


Figure 2

Fine root mass density at 0–10, 10–20 and 20–30 cm depth in control and exclusion plots at sites 5 and 7 (a) in 2024, during throughfall exclusion, and (b) in 2025, the year after exclusion ended (post-exclusion). Each point is one subplot (two per plot and depth), slightly offset vertically so that close values remain visible, and ticks and bars show the mean ± SD of the two subplots

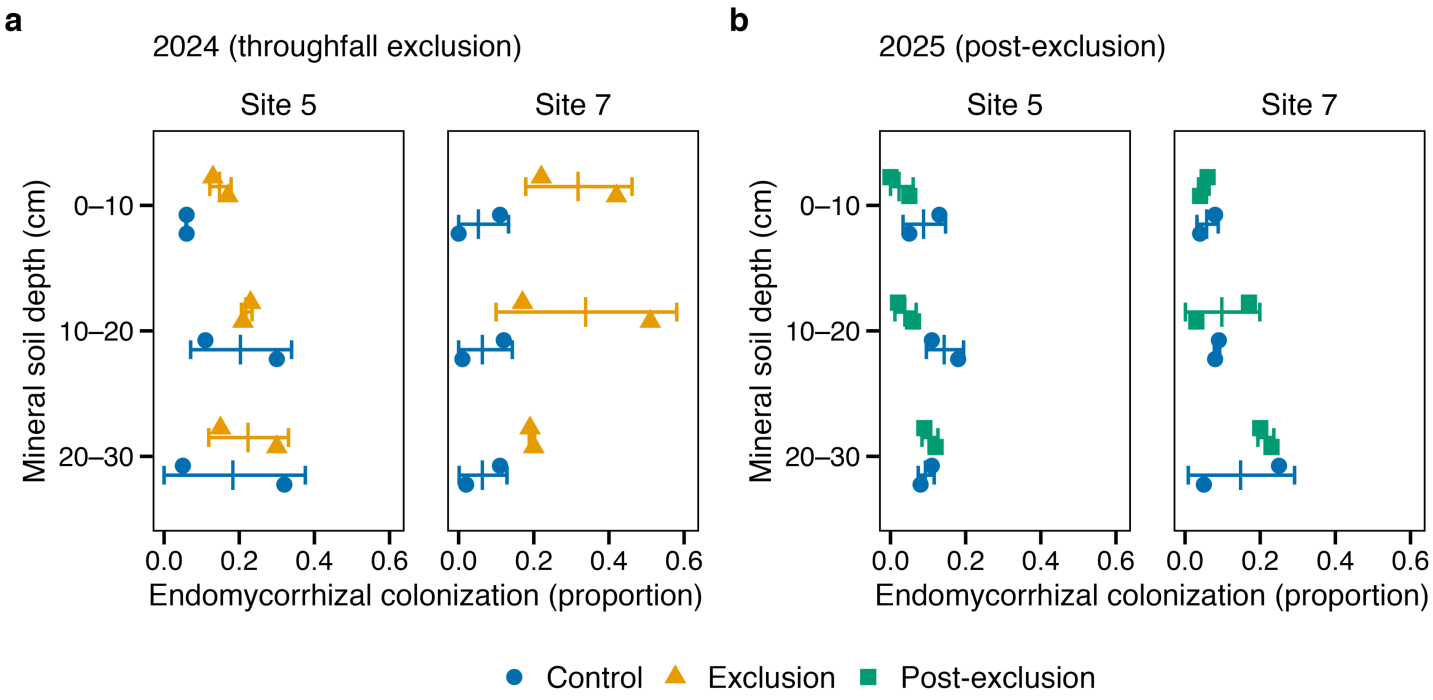


Figure 3

Endomycorrhizal colonization of fine roots (proportion of 100 root-gridline intersections colonized) at 0–10, 10–20 and 20–30 cm depth in control and exclusion plots at sites 5 and 7 (a) in 2024, during throughfall exclusion, and (b) in 2025, the year after exclusion ended (post-exclusion). Each point is one subplot (two per plot and depth), slightly offset vertically so that close values remain visible, and ticks and bars show the mean ± SD of the two subplots (lower bars truncated at zero)

Supplementary Files

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- ESM1.pdf