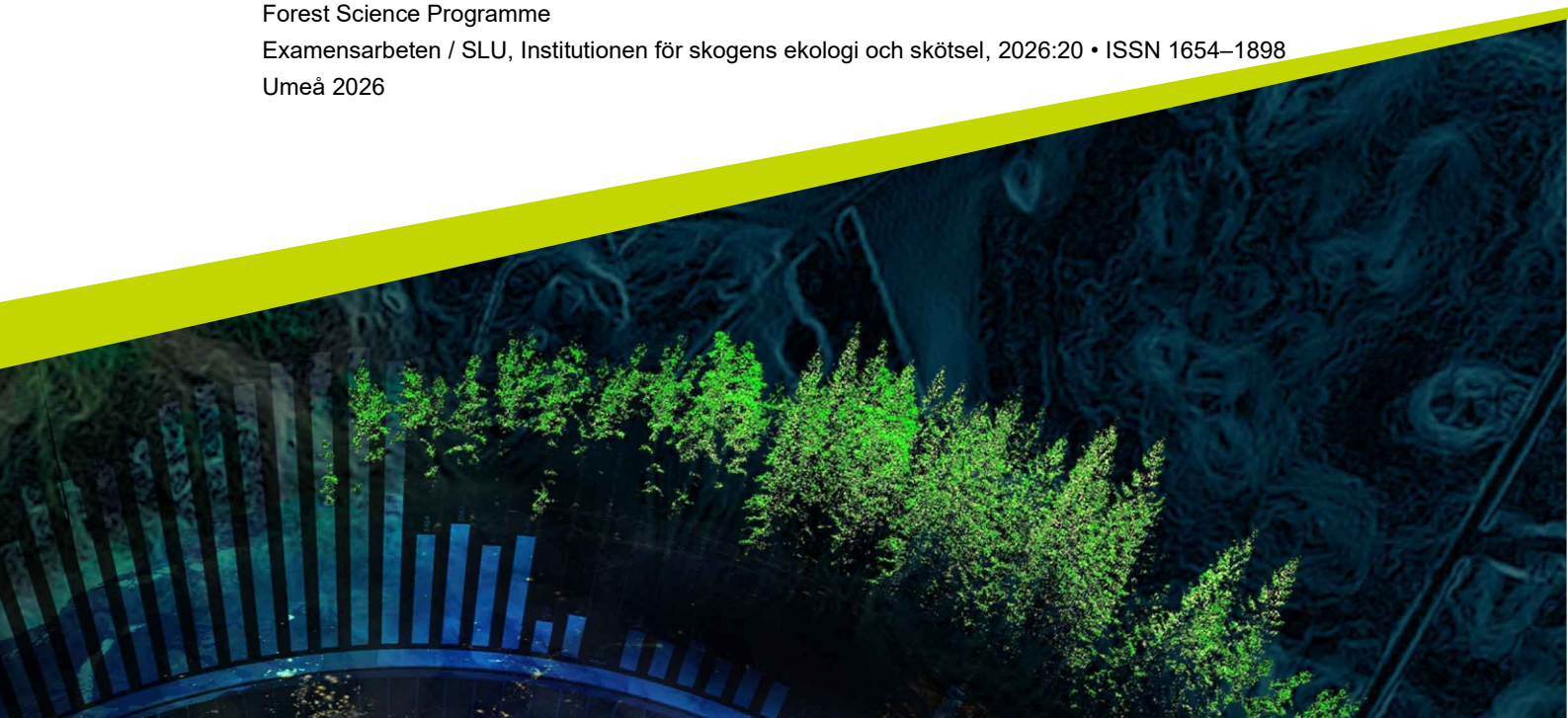
Interactive effects of warming and water regime on belowground strategies of Scots pine and Norway spruce seedlings

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Abstract

In this study, we cultivated Scots pine and Norway spruce seedlings in climate chambers that simulated the temperatures experienced during the growing season in northern forests. The experiment included ambient and warmed (+4 °C) temperature regimes, wet (designed as 70% of operational water-holding capacity) and drought (designed as 40% of operational water-holding capacity) treatments and an additional treatment involved returning warmed, drought-exposed seedlings were returned to the wet target. The results showed that warming increased total biomass and reduced the root-to-shoot ratio. Water treatment had no significant effect on total biomass. Warming reduced RTD, whereas drought increased it, and these effects did not differ significantly between species. Specific root length (SRL) responses to temperature and soil moisture differed between species and among temperature–moisture combinations. Moisture effects on ectomycorrhizal (ECM) colonization were similarly dependent on species and temperature: under warming, drought significantly reduced ECM colonization in Norway spruce, while Scots pine generally maintained high colonization. Following rewatering, RTD decreased in both species to values indistinguishable from those in the wet treatment. In Norway spruce, SRL and ECM colonization increased relative to continued drought but remained significantly lower than under continuously wet conditions. These belowground changes were detected despite the absence of significant moisture-related differences in total biomass. The lack of detectable difference in biomass may mask changes in the belowground resource-acquisition structures of seedlings.

Keywords: boreal forest, root traits, climate chamber experiment, drought, global warming, mycorrhizal colonization, root economics spectrum, *Pinus sylvestris*, *Picea abies*

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Abbreviations

Abbreviation	Description
RTD	Root tissue density
SRL	Specific root length
ECM	Ectomycorrhizal
ANOVA	Analysis of variance
PCA	Principal component analysis
HC3	Heteroscedasticity-consistent covariance matrix estimator, type 3
RES	root economics spectrum
WHC	Water-holding capacity

AI declaration

During the preparation of this thesis, the author used ChatGPT (OpenAI) to assist with text drafting and revision, and the development and revision of R code. The author reviewed and edited all text produced by the tool and takes full responsibility for the final content of the thesis.

1. Introduction

Climate change is altering both temperature and water availability around the world, with consequences for the functioning and persistence of terrestrial ecosystems (Lee et al. 2023). Global mean temperature has already increased substantially, while changes in precipitation patterns, soil moisture and the hydrological cycle are modifying the amount and seasonal availability of water to terrestrial ecosystems. At the same time, heatwaves and droughts are becoming more frequent or intense in many regions, and the likelihood of compound heat and drought events is projected to increase with further warming (Clarke et al. 2022; Lee et al. 2023). Because temperature and water availability can jointly regulate plant growth and survival, understanding their interactive effects is important for predicting ecosystem responses to future climate change.

Boreal forests are increasingly affected by rising temperatures and changing precipitation patterns (Reich et al. 2022). In northern regions, warming and earlier snowmelt may advance spring carbon uptake, lengthen the growing season and promote forest growth (Myneni et al. 1997). Climatic warming has been associated with increased annual forest growth in Finland, while earlier snowmelt has advanced the spring recovery of carbon uptake across the Northern Hemisphere's boreal evergreen forests (Kauppi et al. 2014; Pulliainen et al. 2017). However, there are also concerns that these benefits may be conditional and transitory. In eastern Canada's boreal forest, moderate warming was projected to enhance forest productivity; however, further warming may reverse this response, particularly when accompanied by reduced water availability (D'Orangeville et al. 2018). Higher temperatures can increase evapotranspiration and atmospheric water demand, thereby intensifying water stress even where precipitation does not decline (Wang et al. 2014; D'Orangeville et al. 2018). Increasing atmospheric dryness has reduced the growth of major boreal tree species across Canada, while regional drought has been associated with widespread increases in tree mortality (Peng et al. 2011; Mirabel et al. 2023).

Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) are the two dominant conifer species in the Fennoscandian boreal forest (Esseen et al. 1997). They also have considerable economic importance for the wood market in Europe (Aldea et al. 2021). Scots pine is a light-demanding pioneer species, while Norway spruce is a late-successional, moderately shade-tolerant species which is often considered to be more sensitive to drought than Scots pine (Robakowski 2005; Niinemets & Valladares 2006; Aldea et al. 2021). Given the ongoing climate change, both species are expected to face altered temperature and moisture regimes that may influence their growth performance. In Sweden, it is

predicted that Norway spruce may be more susceptible under a warmer and drier environment, while Scots pine may benefit from it (Aldea et al. 2024). However, during the early stages of establishment, rising temperatures may increase the risk of drought, evaporative demand and intensify the effects of declining water availability, thereby impairing the growth and survival of both Pinus sylvestris and Picea abies seedlings and potentially constraining successful forest regeneration (Matías & Jump 2014; Seidel et al. 2019; Húdoková et al. 2023; Domevscik 2024).

Naturally regenerated seedlings of Scots pine and Norway spruce are particularly vulnerable during their first years of establishment (Nilsson et al. 2002). At this stage, seedlings have limited biomass and carbon reserves, and newly assimilated carbon must be allocated among aboveground growth, root-system development, respiration and tissue maintenance, and carbon supply to symbiotic microorganisms (Pumpanen et al. 2009; Heinonsalo et al. 2010). Environmental changes may therefore affect both the amount of biomass accumulated by seedlings and its allocation among competing functions.

As the roots of seedlings are small and still developing, the volume of soil they can directly explore is limited. How seedlings develop and maintain their root systems is therefore particularly important for successful establishment (Aaltonen et al. 2017; Luoranen 2018). Root functional traits provide measurable indicators of how plants construct their root systems and acquire soil resources (Freschet et al. 2021; Yu et al. 2025), while root morphology and tissue characteristics are closely related to nutrient uptake (Barber & Silberbush 1984; Wang et al. 2006). Plants can modify their capacity for soil exploration through changes in root diameter, root branching and the spatial distribution of fine roots (Kong et al. 2014; Kulmatiski et al. 2020). However, different components of the root phenotype do not necessarily respond in parallel to environmental change. A global meta-analysis found that experimental warming generally increased fine-root biomass and production, whereas specific root length and root diameter showed no consistent overall responses (Wang et al. 2021a). Similarly, a 60-day drought experiment found relatively modest changes in the root architecture of Scots pine and Norway spruce seedlings (Niemczyk et al. 2023). A cross-climate study of Norway spruce found no clear latitudinal plasticity in root tissue density (RTD; root dry mass per unit root volume), whereas specific root length (SRL; root length per unit root dry mass), total root length and root diameter differed significantly among study locations (Ostonen et al. 2013). Environmental changes may affect root biomass, overall size, morphology and tissue architecture independently, and the direction and magnitude of the responses of these components are not necessarily consistent. Changes in a single root parameter are insufficient to fully characterise the underground architecture and resource

acquisition strategies of seedlings. And a combination of multiple root functional traits is needed for analyzing the seeding growth performance.

In addition to investing carbon in their own root systems, Scots pine and Norway spruce seedlings commonly form symbiotic associations with ectomycorrhizal (ECM) fungi (Velmala et al. 2014; Rudawska et al. 2018; Hamberg et al. 2024). Host plants supply assimilated carbon to their fungal partners, while the fungi form mycorrhizal structures and extraradical hyphal networks that extend beyond the soil volume directly accessible to roots and acquire water and mineral nutrients for the host plant (Peterson et al. 2004). Seedlings may therefore acquire belowground resources either directly by developing their own root systems or indirectly by collaborating with ECM fungi to extend their effective range of soil exploration. Both pathways require investment from a limited plant carbon pool and may be adjusted in response to environmental change. The proportion of root tips colonised by mycorrhizae, or the absolute number of mycorrhizal root tips, is often used as an indicator of the relationship between plants and fungi. Short-term or moderate drought has sometimes had little effect on ECM colonization in pine seedlings (Kipfer et al. 2012; Moser et al. 2015), whereas prolonged drought has reduced colonization in pine and spruce seedlings (Nilsen et al. 1998; Musella et al. 2025). In contrast, soil warming has been associated with an increase in the number of ECM root tips in pine and spruce (Domisch et al. 2002; Leppälammi-Kujansuu et al. 2013).

These studies suggest that the response of ECM colonization to environmental changes may also vary depending on the treatment, the intensity of drought and its duration. Biomass, root functional traits and ECM colonization respectively reflect the overall growth performance of seedlings, their own root system development and the acquisition of resources facilitated by fungi; a comprehensive analysis of these indicators is required to gain a more complete understanding of how seedlings adapt their response to environmental changes.

To interpret the observed variation in seedling root traits and ectomycorrhizal colonization, the root economics spectrum (RES) and its multidimensional extension provide a useful theoretical framework. One of the basic principles of trait-based plant ecology is the trade-off between rapid resource acquisition and growth on the one hand, and resource conservation and persistence on the other(Wright et al. 2004). This concept underlies the one-dimensional leaf economics spectrum and was subsequently extended to roots through the root economics spectrum (RES) hypothesis (Weemstra et al. 2016). The classical RES proposes that root traits covary along a single gradient ranging from an acquisitive strategy to a conservative strategy (Bardgett et al. 2014; Bergmann et al. 2020).

Among the fine-root traits commonly examined in studies testing the classical RES are SRL, RTD, root diameter and root nitrogen concentration (Weemstra et al. 2016). Under the classical one-dimensional RES hypothesis, root traits related to morphology, metabolism and tissue persistence were expected to covary along a common acquisitive–conservative axis (Reich 2014; Weemstra et al. 2016). For example, higher SRL combined with lower RTD is generally associated with a relatively acquisitive strategy, as greater root length can be constructed per unit biomass with relatively low investment in root tissue. Conversely, low SRL combined with high RTD is associated with a more conservative strategy, characterized by greater investment in root tissue and potentially greater tissue persistence(Ryser 1996; Ostonen et al. 2007; Roumet et al. 2016).

However, numerous studies have shown that variation in root traits cannot be fully explained by a one-dimensional 'fast–slow' economics spectrum. For example, SRL and RTD are often only weakly or non-significantly correlated, suggesting that different dimensions of belowground resource-acquisition strategies can vary independently (Weemstra et al. 2016; Kong et al. 2019). Recent research has therefore proposed that the strategies employed by plants belowground may be structured along two relatively independent gradients. The first of these is a resource-conservation gradient ranging from rapid metabolism to tissue conservation. The second is a collaboration gradient reflecting variation in how plants explore the soil, i.e., whether they rely on their own root systems or the help of mycorrhizal fungi (Bergmann et al. 2020).

In this experiment, we investigated how Scots pine and Norway spruce seedlings respond to contrasting temperature and moisture conditions by examining growth and biomass allocation, root functional traits, and ECM colonization. We hypothesized:

(1) Warming would enhance seedling growth under sufficient water availability, whereas drought would weaken this response, particularly in Norway spruce, and increase relative biomass allocation to roots.

(2) Drought would shift root traits towards a more conservative strategy, with higher RTD and lower SRL, whereas warming under well-watered conditions would cause the opposite pattern.

(3) Drought would reduce ECM colonization, particularly under warming and in Norway spruce. We further expected drought-recovery seedlings under warmed conditions to shift towards continuously well-watered seedlings in in root traits and ECM colonization.

2. Material & Method

2.1 Plant material and seed germination

Seeds of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst.) were used in this study. Pine seeds were obtained from Skaholma (Svenska Skogsplantor), whereas spruce seeds were obtained from the Lillpite FP-7 seed orchard (Svenska Skogsplantor). Before sowing, seeds were surface-sterilized in 3.5% sodium hypochlorite for 5 min. After sterilization, the seeds were rinsed thoroughly under running tap water until the smell of sodium hypochlorite had completely dissipated. The seeds were then soaked in sterilized double-distilled water for approximately 15 h, with the water replaced after the first 2 h. Seeds were germinated at room temperature in culture boxes filled with autoclaved sand. The sand was sprayed with sterilized deionized water to maintain moisture. During the germination period, seedlings were sprayed with sterilized water daily to keep the substrate moist. No fertilizers were applied. The germination period lasted approximately one month.

2.2 Soil collection and preparation

The soil used in this study was collected from Åheden, approximately 60 km north of Umeå, Sweden (64°13′56.24″ N, 19°46′50.85″ E, 180 m a.s.l.). The region has a mean annual temperature of + 2 °C and a mean annual precipitation of 614 mm. The site is located within a pine-dominated forest on a pine moor underlain by fine-grained sorted sediment. The forest stand consists of approximately 80% *Pinus sylvestris*, 15% *Picea abies*, and 5% *Betula* spp. with an average stand age of ~130 years. Soil was sampled from the organic layer and the underlying mineral soil. The collected soil was homogenized and sieved to remove large roots and stones before being used as the growth substrate.

2.3 Experimental design and growth conditions

The growth-chamber experiment consisted of 80 seedlings in total and included two species, Pinus sylvestris and Picea abies, and two temperature treatments, ambient and warmed. The factorial part of the experiment included two target soil-moisture treatments: wet and drought, corresponding respectively to 70% and 40% of the soil water-holding capacity (WHC).

Water-holding capacity (WHC) was operationally defined as the amount of water retained by the soil after saturation and 24 h of free drainage. The oven-

dried soil mass in each pot, determined after drying at 70 °C for 72 h, was approximately 400 g. After saturation and drainage, the wet soil mass was approximately 750 g, corresponding to approximately 350 g of retained water and a gravimetric water content of 0.875 g H_2O g^{-1} oven-dried soil at 100% WHC. The target total pot mass was calculated as:

$$M\,target = M\,soil + M\,pot + f \times (M\,wetsoil - M\,soil)$$

where $M\mathrm{pot}$ is the mass of the empty pot (60 g), $M\mathrm{soil}$ is the dry soil mass, $M\mathrm{wetsoil}$ is the soil mass after saturation and 24 h of free drainage, and f is 0.70 for the wet treatment or 0.40 for the drought treatment.

The resulting target gravimetric water contents were approximately 0.613 g H_2O g^{-1} dry soil (61.3%) in the wet treatment and 0.350 g H_2O g^{-1} dry soil (35.0%) in the drought treatment. These values corresponded to total target pot masses of approximately 705 g and 600 g, including an empty-pot mass of approximately 60 g. Pots were weighed every three days and watered back to their respective target masses.

The warming treatment was set at +4 °C relative to the ambient temperature treatment (see below). Each treatment combination included eight replicate seedlings. blished under warmed conditions only. For each species, eight additio Together, this resulted in 2 species (pine, spruce) × 2 temperature treatments (warmed, ambient) × 2 moisture treatments (70% and 40%) × 8 replicates = 64 experimental units. In addition to this factorial design, a drought-recovery treatment was esta nal seedlings were initially exposed to the warmed-drought treatment and were subsequently returned to wet conditions. Thus, the drought-recovery treatment comprised 2 species × 1 temperature treatment × 1 recovery treatment × 8 replicates = 16 additional experimental units. Together, the entire experiment comprised a total of 64 + 16 = 80 experimental units.

The growth chamber experiment was established on 6 January 2026. Seedlings with similar growth performance were selected from the germination boxes. Their roots were gently rinsed with deionized water, and the seedlings were then carefully transplanted into pots (9 × 9 × 20 cm) filled with the prepared soil substrate. The pots were placed in two growth chambers corresponding to the ambient and warmed temperature treatments.

The temperature settings and photoperiod in the growth chambers were determined based on meteorological data from the Svartberget Research Station and Experimental Forest (close to the soil collection site), for the period from 1 June to 15 September during 2022–2024 (*Meteorological data from Svartberget,*

Åheden AWS, 2022–2024 2025). Temperatures were averaged across 3 years for each day of the growing-season period, and second-order polynomial regressions were fitted separately to the daily maximum and minimum temperatures. The fitted daily temperatures were converted into weekly light- and dark-period settings using the calculated photoperiod at 64.25° N. The warmed treatment was maintained at 4 °C above the corresponding ambient setting. Further details and the resulting weekly chamber settings are provided in the Appendix.

Due to the technical limitations of the growth chambers, one light-period temperature and one dark-period temperature were used per day, and these settings were adjusted weekly. Relative humidity in both chambers was set at 90%. Photosynthetically active radiation was set to 200 μmol m^{-2} s^{-1}, and the red: far-red ratio was set to 1:1. To minimize potential chamber effects, the two chambers were alternated weekly between ambient and warming conditions, and the positions of seedlings within each chamber were also rotated.

During the first month after transplantation, all seedlings were maintained under wet conditions. Pots were weighed and watered every three days to maintain the target moisture level. The drought treatment started on 6 February 2026. For seedlings assigned to the drought treatment, watering was suspended until the pot weight had decreased to the target drought level. Thereafter, pots were watered every three days to maintain the target moisture level. Seedlings in the drought-recovery treatment group remained at 40% of operational WHC for approximately five weeks, until 15 March 2026, after which they were returned to the wet condition of 70% of operational WHC. Seedlings assigned to the drought treatment groups were maintained at 40% of operational WHC until the end of the experiment.

2.4 Harvest and trait measurements

Seedlings were harvested on 23 April 2026, 15 weeks after the start of the experiment. Seedlings were separated into roots, stems and needles. First, for each seedling, roots were gently washed free of soil and scanned at 600 dpi using an Expression 13000xl scanner. Root morphological traits, including total root length, average root diameter, root surface area, root volume and number of root tips, were analyzed from the scanned root images using RhizoVision Explorer 2.0.3.0.

After scanning, the root system of each seedling was visually examined under a microscope to estimate mycorrhizal colonization rates. For each root system, the proportion of root tips colonized by mycorrhizal fungi was estimated using 100 grid-root intersections, following the approach of Kuťáková et al. (2026). Root

tips were classified as colonized when visible mycorrhizal structures, such as a mantle or fungal hyphae, were observed.

After estimating the colonization rate, leaves, stems and roots were dried at 68 °C for 72 h and weighed separately to determine dry biomass. Total biomass was calculated as the sum of leaf, stem and root dry mass. Root-to-shoot ratio was calculated as root dry mass divided by aboveground dry mass. Root tissue density (RTD; g cm^{-3}) was calculated as root dry mass divided by root volume estimated from the scanned images. Specific root length (SRL; m g^{-1}) was calculated as total root length estimated from the scanned images divided by root dry mass.

2.5 Statistical analyses of treatment effects

2.5.1 ANOVA test

All statistical analyses were conducted in R version 4.4.2. Individual seedlings were treated as the experimental units. Three complementary model structures were used to evaluate treatment effects.

Analysis 1 examined the effects of temperature, moisture, species and all their interactions. It included the ambient–drought, ambient–wet, warmed–drought and warmed–wet treatments, whereas the warmed–recovery treatment was excluded because the experiment did not include ambient-recovery treatment. This analysis included 64 seedlings, with eight seedlings in each species × temperature × moisture combination.

Analysis 2 examined the effects of moisture status, species and their interaction within the warmed treatments, including warmed drought, warmed wet and warmed recovery. This analysis included 48 seedlings, with eight seedlings in each moisture-status × species combination.

For completeness, an additional exploratory analysis treating the five temperature–moisture combinations as distinct experimental scenarios was conducted. This analysis included all 80 seedlings and fitted scenario, species and their interaction as explanatory variables and the result is reported in the Appendix.

Before inferential testing, the suitability of analyzing each response variable on its original or transformed scale was evaluated. For positive continuous variables, models fitted to the original and natural-log-transformed values were compared using residual Q–Q plots, residual-versus-fitted plots, Shapiro–Wilk tests of model residuals and Box–Cox profile-likelihood estimates. The final

transformation was selected from this combined evidence. Ectomycorrhizal colonization rates, which were expressed as proportions, were logit-transformed before analysis. The transformation applied to each response variable is reported in the corresponding results table.

Variance homogeneity was assessed using Levene tests across the treatment combinations represented in each model. To reduce sensitivity to remaining heteroscedasticity, all final models were evaluated using Type II analysis of variance with heteroscedasticity-consistent HC3 covariance matrices, implemented using the car package. The same HC3 covariance matrices were supplied to subsequent estimated-marginal-mean analyses conducted using the emmeans package. Where multiple pairwise or planned contrasts were required, their p values were adjusted using Holm's sequential procedure within each prespecified family of comparisons. Statistical significance was evaluated at $\alpha = 0.05$.

2.5.2 Correlations among root traits

Guided by the bivariate trait relationships proposed in the root economics space framework of Bergmann et al. (2020), we examined whether the predicted associations were detectable among individual seedlings within each species. As the root biomass occurs in the denominator of SRL and the numerator of RTD and may contribute mathematically to the observed SRL-RTD relationship, the biomass-related correlations were included. The first set of analyses comprised five variable pairs: diameter–SRL, SRL–RTD, RTD–diameter, root biomass–SRL and root biomass–RTD. The second set examined the relationships of ectomycorrhizal colonization rate with average root diameter, SRL and RTD.

Spearman rank correlations were calculated separately for Scots pine and Norway spruce. Raw correlations described the overall associations across all five experimental scenarios, whereas scenario-adjusted partial Spearman correlations were used to estimate trait covariation after differences among scenario means had been removed. For the latter, each variable was first converted to ranks and regressed separately against scenario as a categorical factor; the residuals from the two models were then correlated.

To control the family-wise error rate, Holm correction was applied independently to the raw and scenario-adjusted correlations within each analysis set. The correction therefore covered ten tests for the root-trait and biomass analyses (five variable pairs × two species) and six tests for the colonization analyses (three variable pairs × two species). Spearman correlation coefficients

(r_s), unadjusted *p*-values and Holm-adjusted *p*-values are reported, with statistical significance assessed from the Holm-adjusted values at $p = 0.05$.

3. Results

3.1 Biomass variables

In Analysis 1, total biomass, needle biomass, stem biomass, and root-to-shoot ratio were significantly affected by temperature and species, but not by moisture, and none of their interaction terms was significant (Table 1). Root biomass showed significant effects of temperature and species and a significant temperature × moisture × species interaction, while the moisture main effect and all two-way interactions were nonsignificant. Within the warmed treatments in Analysis 2, total, needle, stem, and root biomass were not significantly affected by moisture status, and none of their moisture status × species interactions was significant (Table 2).

Table 1. Effects of temperature, moisture, and species on seedling biomass components and biomass allocation. Values are F statistics from Type II Wald tests with HC3 correction, with p values in parentheses. Significant effects ($p < 0.05$) are shown in bold. Variables marked "log" were log-transformed before analysis, whereas "raw" indicates that untransformed data were analyzed. The drought-recovery treatment was excluded. T, temperature; M, moisture; S, species.

Response variable	Transformation	Temperature	Moisture	Species	T × M	T × S	M × S	T × M × S
Total biomass	log	25.70 **(<0.001)**	0.45 (0.507)	**32.84 (< 0.001)**	0.13 (0.721)	0.73 (0.398)	0.02 (0.899)	0.90 (0.346)
Needle biomass	raw	27.62 **(<0.001)**	2.12 (0.151)	**32.63 (< 0.001)**	0.14 (0.714)	2.09 (0.153)	0.11 (0.744)	0.33 (0.568)
Stem biomass	log	12.09 **(<0.001)**	0.01 (0.904)	**4.38 (0.041)**	0.02 (0.876)	3.88 (0.054)	0.11 (0.747)	0.00 (0.989)
Root biomass	log	16.08 **(<0.001)**	0.03 (0.866)	**37.04 (< 0.001)**	0.03 (0.859)	1.26 (0.266)	0.36 (0.552)	**4.42 (0.040)**
Root-to-shoot ratio	log	18.77 **(<0.001)**	3.18 (0.080)	**6.24 (0.015)**	1.59 (0.213)	0.03 (0.857)	0.01 (0.923)	2.36 (0.130)

Table 2. Effects of moisture status and species on seedling biomass components and biomass allocation under warmed conditions. The analysis included the warmed–drought, warmed–wet, and warmed–recovery treatments. Values are F statistics from Type II Wald tests with HC3 correction, with p values in parentheses. Significant effects ($p < 0.05$) are shown in bold. Variables marked "log" were log-transformed before analysis, whereas "raw" indicates that untransformed data were analyzed.

Response variable	Transformation	Moisture status	Species	Moisture status × Species
Total biomass	log	0.03 (0.972)	**16.70 (< 0.001)**	0.19 (0.831)

Response variable	Transformation	Moisture status	Species	Moisture status × Species
Needle biomass	raw	0.10 (0.908)	**27.39 (< 0.001)**	0.22 (0.800)
Stem biomass	log	0.02 (0.977)	2.25 (0.141)	0.61 (0.548)
Root biomass	log	0.03 (0.966)	**15.34 (< 0.001)**	0.86 (0.432)
Root-to-shoot ratio	log	0.06 (0.939)	3.09 (0.086)	1.04 (0.362)

Across species and moisture treatments, warming significantly increased total, needle, and stem biomass and decreased the root-to-shoot ratio (Fig. 1). None of the interaction terms was significant for these variables. Scots pine had greater average total and needle biomass than Norway spruce, whereas Norway spruce had a higher average root-to-shoot ratio.

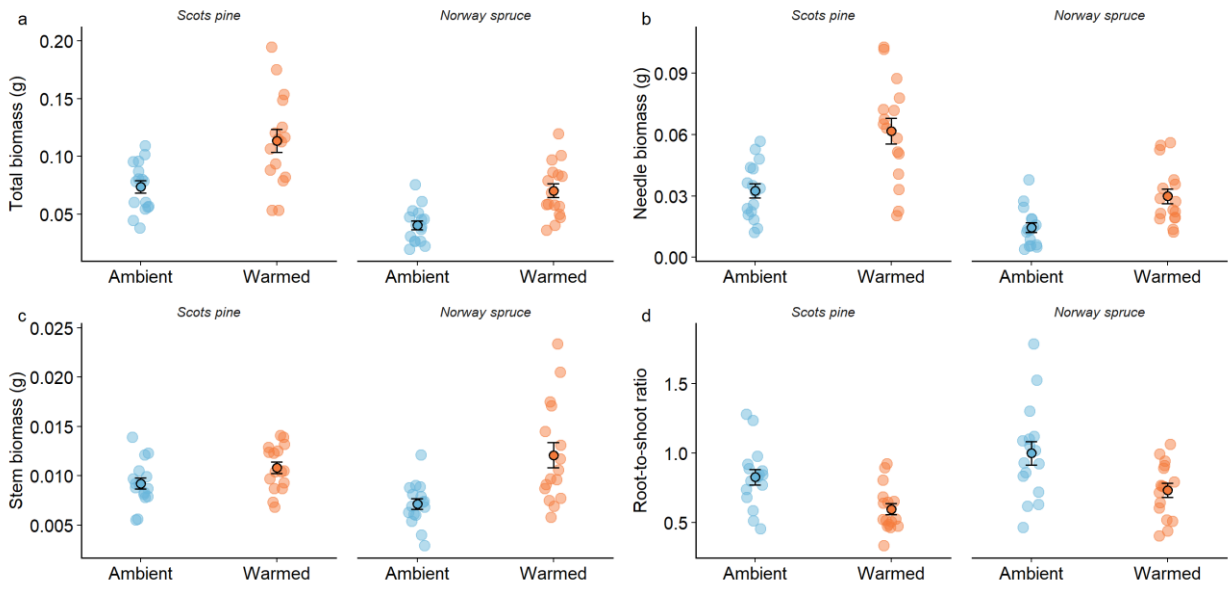


Figure 1. Effects of temperature on seedling biomass and biomass allocation in Scots pine and Norway spruce. (a) Total biomass, (b) needle biomass, (c) stem biomass, and (d) root-to-shoot ratio under ambient and warmed temperature conditions, averaged across drought and wet treatments within each species. Recovery-treatment seedlings are excluded. Small points represent individual observations, and large points with error bars represent means ± SE. All values are presented on the original scale.

For root biomass, subsequent species-specific analyses showed that root biomass increased significantly with warming in Norway spruce, whereas the corresponding temperature effect in Scots pine did not reach statistical significance (Fig. 2). The warming-associated difference in mean root biomass was smaller under drought than under wet conditions in Scots pine, whereas the

opposite pattern occurred in Norway spruce (Fig. 2). But the temperature × moisture interaction was not significant in either species.

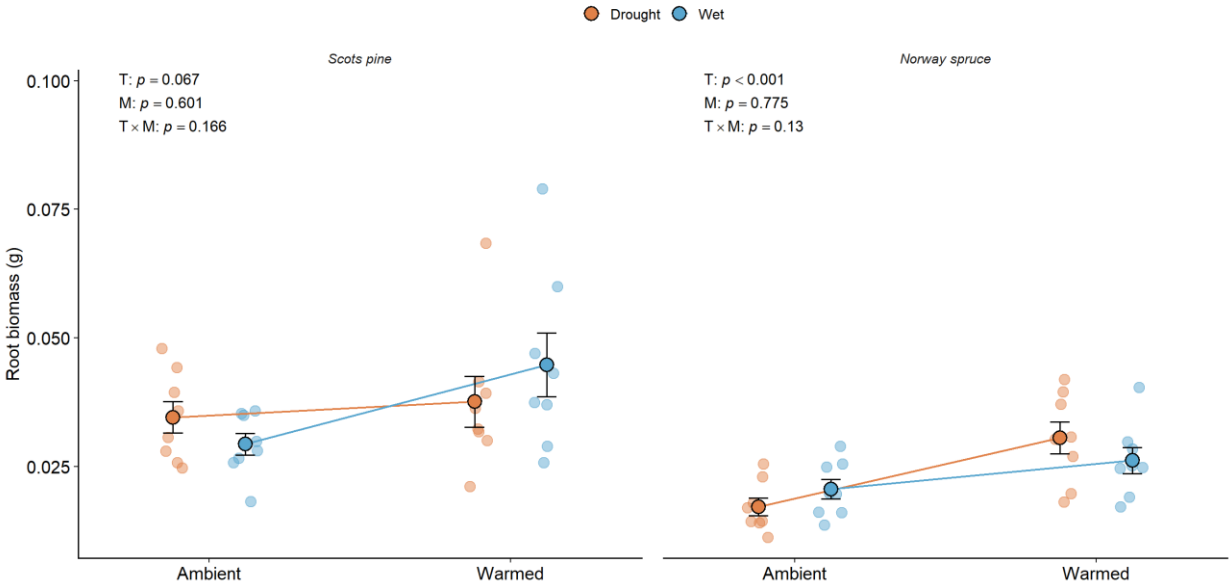


Figure 2. Effects of temperature and water treatment on root biomass in Scots pine and Norway spruce seedlings. Root biomass is shown under ambient and warmed temperatures for drought and wet treatments, separately for each species. Recovery-treatment seedlings are excluded. Small points represent individual observations, and large points with error bars represent means ± SE. Lines connect means for the same moisture treatment across temperatures. The displayed p-values are from species-specific models testing the effects of temperature (T), moisture (M), and their interaction (T × M). All values are presented on the original scale.

3.2 Root traits

In Analysis 1, total root length, root-tip number, and root tissue density (RTD) were significantly affected by temperature, moisture, and species, with no significant interaction terms (Table 3). Average root diameter was significantly affected by moisture and species and showed a significant temperature × species interaction, but the temperature main effect and the remaining interactions were nonsignificant. Specific root length (SRL) showed a significant temperature × moisture × species interaction, significant temperature and moisture main effects and a significant temperature × species interaction.

Within the warmed treatments in Analysis 2, total root length, root-tip number, and average root diameter were significantly affected by species, but not by moisture status; none of their moisture status × species interactions were significant (Table 4). RTD was significantly affected by species and moisture status, but their interaction effect was not significant. SRL showed a significant moisture status effect and a significant moisture status × species interaction.

Table 3. Effects of temperature, moisture, and species on seedling root morphological and functional traits. Values are F statistics from Type II Wald tests with HC3 correction, with p values in parentheses. Significant effects ($p < 0.05$) are shown in bold. Variables marked "log" were log-transformed before analysis, whereas "raw" indicates that untransformed data were analyzed. The drought-recovery treatment was excluded. T, temperature; M, moisture; S, species; RTD, root tissue density; SRL, specific root length.

Response variable	Transformation	Temperature	Moisture	Species	T × M	T × S	M × S	T × M × S
Average root diameter	raw	3.86 (0.055)	**4.91 (0.031)**	**58.46 (<0.001)**	0.00 (0.992)	**9.85 (0.003)**	3.31 (0.074)	1.67 (0.201)
Total root length	log	**30.06 (<0.001)**	**4.87 (0.031)**	**28.39 (<0.001)**	0.06 (0.805)	0.00 (0.958)	0.55 (0.460)	1.16 (0.285)
Number of root tips	log	**19.55 (<0.001)**	**4.39 (0.041)**	**22.32 (<0.001)**	0.00 (0.966)	0.03 (0.868)	1.26 (0.266)	1.53 (0.221)
RTD	raw	**13.94 (<0.001)**	**48.13 (<0.001)**	**40.46 (<0.001)**	0.43 (0.515)	0.05 (0.825)	0.00 (0.964)	0.25 (0.622)
SRL	log	**35.91 (<0.001)**	**64.42 (<0.001)**	0.29 (0.593)	0.46 (0.502)	**8.96 (0.004)**	1.90 (0.173)	**7.32 (0.009)**

Table 4. Effects of moisture status and species on seedling root morphological and functional traits under warmed conditions. The analysis included the warmed–drought, warmed–wet, and warmed–recovery treatments. Values are F statistics from Type II Wald tests with HC3 correction, with p values in parentheses. Significant effects ($p < 0.05$) are shown in bold. Variables marked "log" were log-transformed before analysis, whereas "raw" indicates that untransformed data were analyzed. RTD, root tissue density; SRL, specific root length.

Response variable	Transformation	Moisture status	Species	Moisture status × Species
Average root diameter	raw	2.60 (0.086)	**25.44 (<0.001)**	2.79 (0.073)
Total root length	log	0.93 (0.404)	**19.66 (<0.001)**	0.12 (0.883)
Number of root tips	log	1.34 (0.274)	**23.90 (<0.001)**	0.13 (0.880)
RTD	raw	**11.47 (<0.001)**	**21.77 (<0.001)**	0.20 (0.821)

SRL	log	**17.08 (<0.001)**	3.30 (0.077)	**4.02 (0.025)**

Total root length and root-tip number showed similar responses to the treatments. The temperature main effect indicated greater total root length and more root tips under warmed than under ambient conditions, while the moisture main effect indicated higher values of both traits under wet than under drought conditions. The species main effect indicated greater total root length and more root tips in Scots pine than in Norway spruce (Table 3; Fig. 3).

For average root diameter, mean values decreased slightly with warming in Scots pine, with a larger decrease under drought than under wet conditions. In Norway spruce, mean diameter increased slightly under drought and remained almost unchanged under wet conditions. Average root diameter showed a significant temperature × species interaction, while subsequent species-specific analyses did not detect a significant temperature effect in either Scots pine or Norway spruce (Fig. 4).

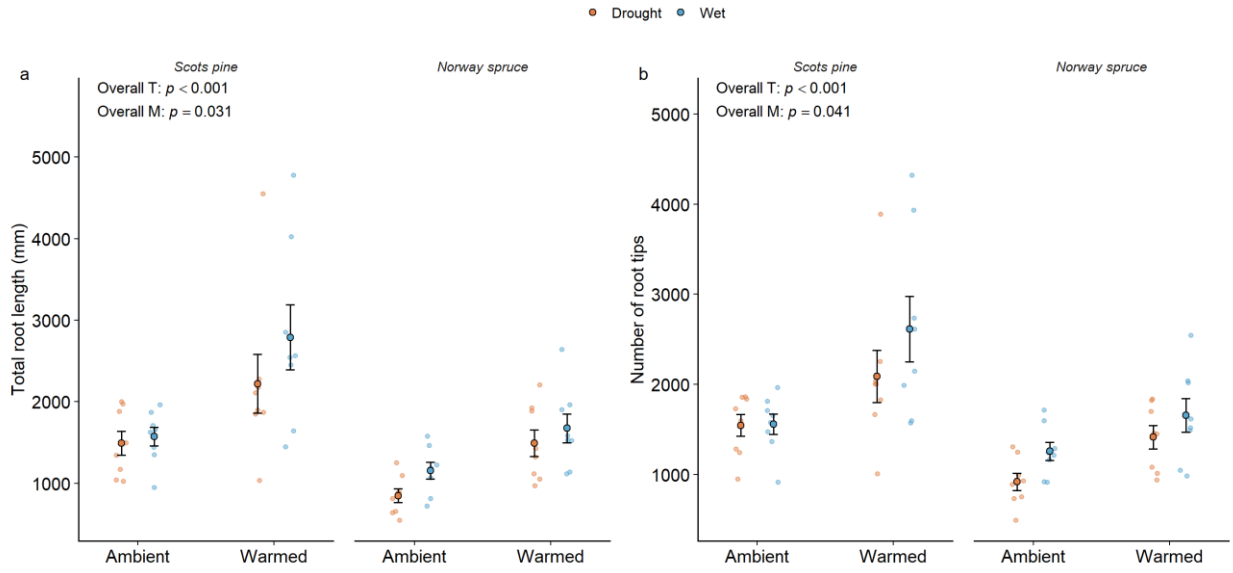


Figure 3. Effects of temperature and water treatment on (a) total root length and (b) number of root tips in Scots pine and Norway spruce seedlings. Values are shown under ambient and warmed temperatures for drought and wet treatments, separately for each species. Recovery-treatment seedlings are excluded. Small points represent individual observations, and large points with error bars represent means ± SE. Orange and blue indicate drought and wet conditions, respectively. The displayed p-values report the overall main effects of temperature (T) and moisture (M); all values are presented on the original scale.

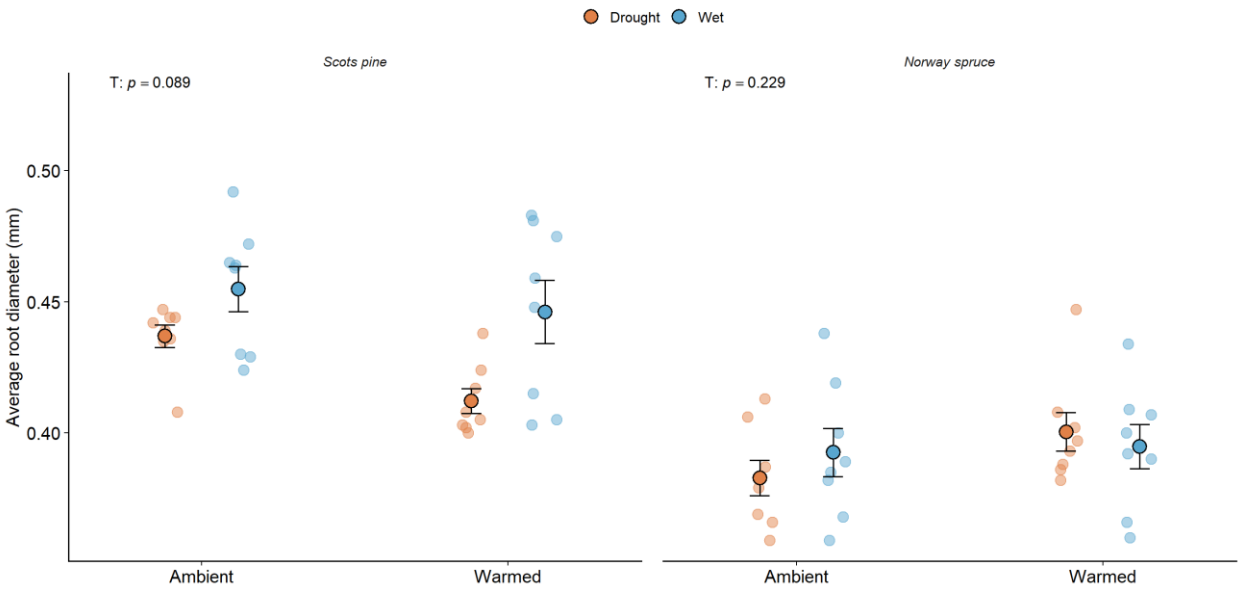


Figure 4. Average root diameter of Scots pine and Norway spruce seedlings under contrasting temperature and water-availability treatments. Values are shown under ambient and warmed temperatures, separately for each species. Following a significant temperature × species interaction in Table 3, the main effect of temperature was examined separately within each species using one-way ANOVAs with HC3 correction, pooling observations across moisture treatments. The corresponding p-values are shown in each panel. Recovery-treatment seedlings are excluded.

For RTD, the temperature main effect indicated lower RTD under warmed than under ambient conditions, while the moisture main effect indicated lower RTD under wet than under drought conditions. The species main effect indicated lower RTD in Scots pine than in Norway spruce (Table 3; Fig. 5a). Within the warmed treatments, comparisons of mean RTD among moisture treatments showed higher RTD under drought than under either wet or recovery conditions (drought vs. wet: Holm-adjusted p = 0.001; drought vs. recovery: Holm-adjusted p < 0.001), whereas wet and recovery conditions did not differ significantly (Holm-adjusted p = 0.601; Fig. 5).

For SRL, comparisons among the four temperature × moisture treatment groups within each species revealed different patterns (Fig. 6). In Scots pine, SRL was significantly lower under ambient-drought conditions than under each of the other three groups (ambient-drought vs. ambient-wet: Holm-adjusted $p < 0.001$; ambient-drought vs. warmed-drought: Holm-adjusted $p < 0.001$; ambient-drought vs. warmed-wet: Holm-adjusted $p < 0.001$). SRL was also significantly higher under warmed-wet than under ambient-wet conditions (Holm-adjusted $p = 0.016$), while warmed-drought did not differ significantly from either ambient-wet or warmed-wet (warmed-drought vs. ambient-wet: Holm-adjusted $p = 0.197$; warmed-drought vs. warmed-wet: Holm-adjusted $p = 0.205$). In Norway spruce, SRL was significantly higher under warmed-wet conditions than under either

ambient-drought or warmed-drought conditions (warmed-wet vs. ambient-drought: Holm-adjusted $p < 0.001$; warmed-wet vs. warmed-drought: Holm-adjusted $p < 0.001$). Ambient-wet did not differ significantly from any of the other combinations (ambient-wet vs. ambient-drought: Holm-adjusted $p = 0.166$; ambient-wet vs. warmed-drought: Holm-adjusted $p = 0.166$; ambient-wet vs. warmed-wet: Holm-adjusted $p = 0.166$).

Within the warmed treatments, SRL did not differ significantly among drought, wet, and recovery conditions in Scots pine (drought vs. wet: Holm-adjusted $p = 0.514$; drought vs. recovery: Holm-adjusted $p = 0.514$; wet vs. recovery: Holm-adjusted $p = 0.805$). In Norway spruce, SRL was highest under wet conditions, intermediate under recovery conditions, and lowest under drought conditions. All three pairwise differences were significant (wet vs. drought: Holm-adjusted $p < 0.001$; wet vs. recovery: Holm-adjusted $p = 0.040$; recovery vs. drought: Holm-adjusted $p = 0.006$; Fig. 6).

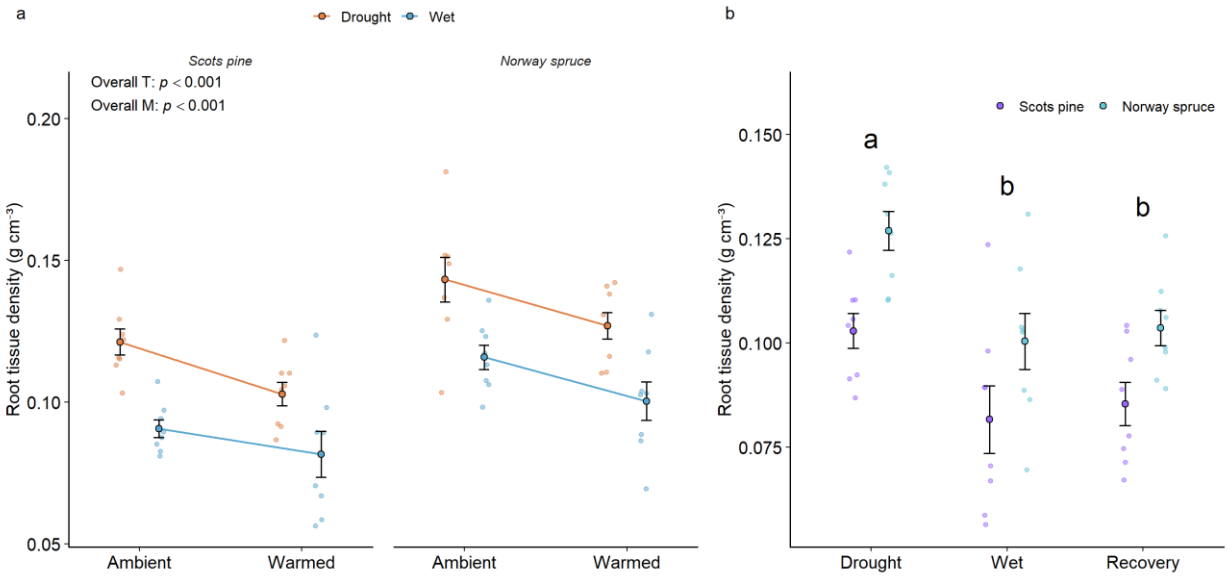


Figure 5. Effects of temperature and water treatment on root tissue density (RTD) in Scots pine and Norway spruce seedlings. (a) RTD under ambient and warmed temperatures for drought and wet treatments, shown separately for each species. Lines connect means for the same moisture treatment across temperatures. The displayed p-values report the overall main effects of temperature (T) and moisture (M) from the three-factor HC3-adjusted ANOVA. (b) RTD under drought, wet, and recovery treatments within the warmed treatments. Different lowercase letters indicate significant differences among moisture treatments, averaged across species, based on Holm-adjusted pairwise comparisons ($p < 0.05$); treatments sharing a letter did not differ significantly. In both panels, small points represent individual observations, and large points with error bars represent means ± SE for each treatment within each species.

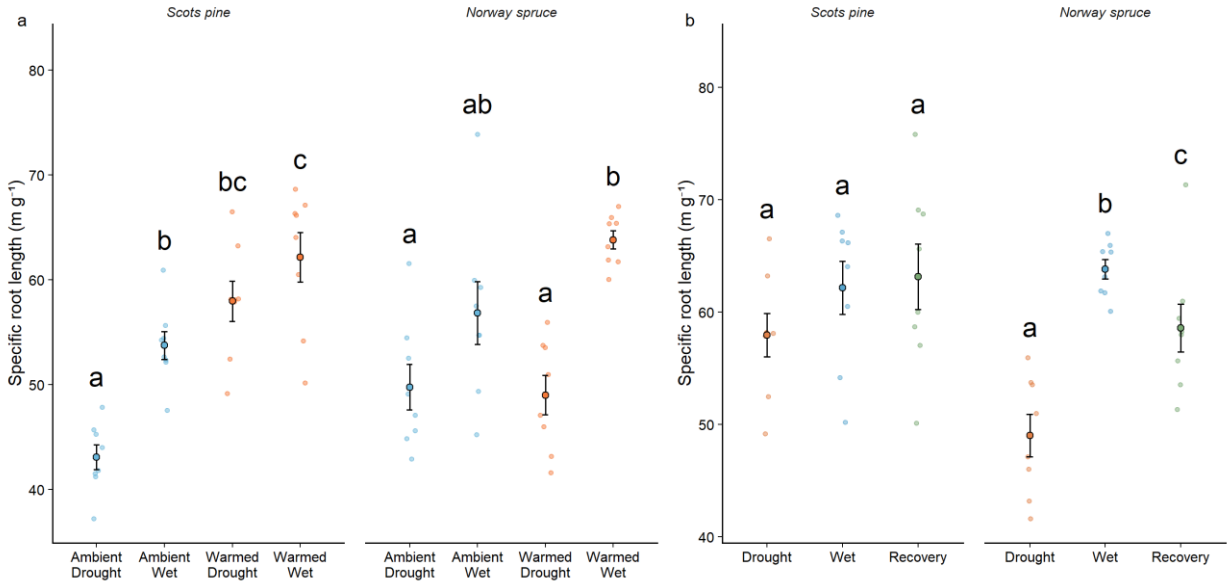


Figure 6. Effects of temperature and water treatment on specific root length (SRL) in Scots pine and Norway spruce seedlings. (a) SRL under the four combinations of ambient or warmed temperatures and drought or wet conditions. (b) SRL under drought, wet, and recovery conditions within the warmed treatments. Both panels show results separately for each species. Small points represent individual observations, and large points with error bars represent means ± SE. Within each species in each panel, different lowercase letters indicate significant differences among treatment combinations ($p < 0.05$); groups sharing at least one letter did not differ significantly.

After controlling for different treatment groups, SRL was negatively correlated with RTD in both Scots pine ($r_s = -0.542$, Holm-adjusted $p = 0.005$) and Norway spruce ($r_s = -0.451$, Holm-adjusted $p = 0.035$). RTD was also negatively correlated with average root diameter in both species (Scots pine: $r_s = -0.673$, Holm-adjusted $p < 0.001$; Norway spruce: $r_s = -0.521$, Holm-adjusted $p = 0.008$). A significant negative association between average root diameter and SRL was detected in Norway spruce ($r_s = -0.438$, Holm-adjusted $p = 0.038$), but not in Scots pine ($r_s = -0.148$, Holm-adjusted $p = 0.778$; Table 5).

Root biomass was negatively correlated with RTD in Scots pine ($r_s = -0.553$, Holm-adjusted $p = 0.004$). No significant correlation was detected in Norway spruce ($r_s = -0.003$, Holm-adjusted $p = 0.984$). Root biomass was not significantly associated with SRL in either Scots pine ($r_s = 0.224$, Holm-adjusted $p = 0.570$) or Norway spruce ($r_s = -0.275$, Holm-adjusted $p = 0.419$). These four biomass-related tests reported the same significance classifications before and after Holm adjustment (Table 5).

Table 5. Scenario-adjusted correlations among root traits in Scots pine and Norway spruce seedlings. Partial Spearman rank correlations were calculated separately for each species while controlling for experimental scenario as a categorical variable. Holm

adjustment was applied jointly across the ten correlation tests. Associations with Holm-adjusted p < 0.05 are shown in bold. Diameter, average root diameter; SRL, specific root length; RTD, root tissue density.

Species	Trait pair	Partial Spearman's r_s	Unadjusted *p*	Holm-adjusted *p*
Scots pine	Diameter–SRL	−0.148	0.389	0.778
Scots pine	SRL–RTD	−0.542	<0.001	**0.005**
Scots pine	RTD–Diameter	−0.673	<0.001	**<0.001**
Scots pine	Root biomass–SRL	0.224	0.190	0.570
Scots pine	Root biomass–RTD	−0.553	<0.001	**0.004**
Norway spruce	Diameter–SRL	−0.438	0.008	**0.038**
Norway spruce	SRL–RTD	−0.451	0.006	**0.035**
Norway spruce	RTD–Diameter	−0.521	0.001	**0.008**
Norway spruce	Root biomass–SRL	−0.275	0.105	0.419
Norway spruce	Root biomass–RTD	−0.003	0.984	0.984

3.3 Colonization rate

In Analysis 1, ectomycorrhizal (ECM) colonization rate was significantly affected by moisture and species and showed significant temperature × species, moisture × species, and temperature × moisture × species interactions. The temperature main effect and the temperature × moisture interaction were nonsignificant (Table 6). Within the warmed treatments in Analysis 2, ECM colonization was significantly affected by moisture status, species and their interaction (Table 6).

Table 6. Type II ANOVA results for ectomycorrhizal colonization rate under two analytical frameworks. Colonization rate was logit-transformed before analysis. Analysis 1 included the factorial combination of temperature, moisture and species, excluding the recovery treatment; Analysis 2 included wet, drought and recovery treatments under warming. Values are HC3-adjusted Type II ANOVA F-statistics and associated p-values. Significant effects (p < 0.05) are shown in bold.

Analysis	Model term	*F*	*p*
Analysis 1: factorial treatments	Temperature	0.65	0.423

Analysis	Model term	F	p
	Moisture	30.20	**< 0.001**
	Species	43.45	**< 0.001**
	Temperature × Moisture	1.68	0.201
	Temperature × Species	4.19	**0.045**
	Moisture × Species	13.18	**< 0.001**
	Temperature × Moisture × Species	15.34	**< 0.001**
Analysis 2: warmed treatments	Moisture status	13.16	**< 0.001**
	Species	11.04	**0.002**
	Moisture status × Species	26.16	**< 0.001**

Comparisons among the four temperature × moisture treatment groups showed different colonization patterns within the two species (Fig. 7). In Scots pine, colonization rate was significantly lower under ambient-drought conditions than under ambient-wet, warmed-drought, or warmed-wet conditions (ambient-drought vs. ambient-wet: Holm-adjusted $p < 0.001$; ambient-drought vs. warmed-drought: Holm-adjusted $p = 0.002$; ambient-drought vs. warmed-wet: Holm-adjusted $p < 0.001$). No significant differences were detected among the latter three groups (ambient-wet vs. warmed-drought: Holm-adjusted $p = 1.000$; ambient-wet vs. warmed-wet: Holm-adjusted $p = 1.000$; warmed-drought vs. warmed-wet: Holm-adjusted $p = 1.000$).

In Norway spruce, colonization rate was significantly lower under warmed-drought conditions than under ambient-drought, ambient-wet, or warmed-wet conditions (warmed-drought vs. ambient-drought: Holm-adjusted $p = 0.024$; warmed-drought vs. ambient-wet: Holm-adjusted $p < 0.001$; warmed-drought vs. warmed-wet: Holm-adjusted $p < 0.001$). Colonization rate under ambient-drought conditions was also significantly lower than under ambient-wet and warmed-wet conditions (ambient-drought vs. ambient-wet: Holm-adjusted $p = 0.017$; ambient-drought vs. warmed-wet: Holm-adjusted $p = 0.006$), while the ambient-wet and warmed-wet groups did not differ significantly (Holm-adjusted $p = 0.376$; Fig. 7).

Within the warmed treatments in Analysis 2, Scots pine had significantly lower colonization rate under recovery conditions than under either drought or wet conditions (recovery vs. drought: Holm-adjusted $p < 0.001$; recovery vs. wet: Holm-adjusted $p < 0.001$), while drought and wet conditions did not differ significantly (Holm-adjusted $p = 0.579$). In Norway spruce, colonization rate was highest under wet conditions, intermediate under recovery conditions, and lowest under drought conditions. All three pairwise differences were significant (wet vs.

drought: Holm-adjusted $p < 0.001$; wet vs. recovery: Holm-adjusted $p = 0.0365$; recovery vs. drought: Holm-adjusted $p < 0.001$; Fig. 7).

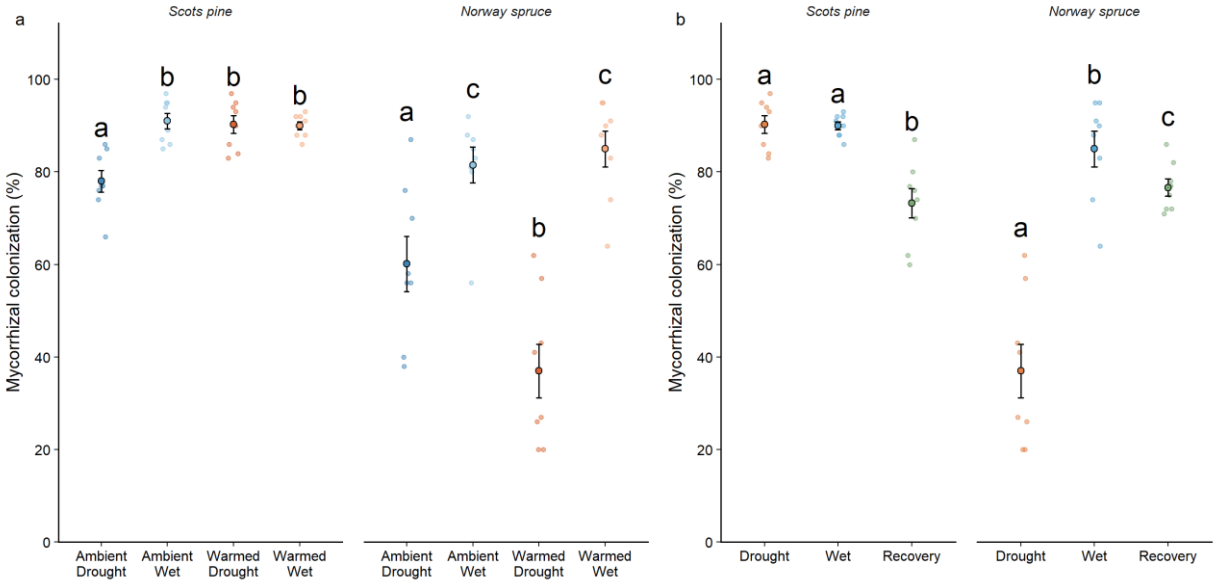


Figure 7. Effects of temperature and water treatment on ectomycorrhizal colonization in Scots pine and Norway spruce seedlings. (a) Colonization under the four combinations of ambient or warmed temperatures and drought or wet conditions, excluding recovery-treatment seedlings. (b) Colonization under drought, wet, and recovery conditions within the warmed treatments. Both panels show results separately for each species. Small points represent individual seedlings, and large points with error bars represent arithmetic means ± SE on the original percentage scale. Within each species in each panel, different lowercase letters indicate significant differences among treatments ($p < 0.05$).

After controlling for experimental scenario, ECM colonization rate was not significantly correlated with average root diameter, SRL, or RTD in Scots pine (diameter: Holm-adjusted $p = 0.979$; SRL: Holm-adjusted $p = 0.979$; RTD: Holm-adjusted $p = 0.979$). In Norway spruce, ECM colonization rate was negatively correlated with RTD (partial Spearman's $r_s = -0.459$, Holm-adjusted $p = 0.0293$), whereas its associations with average root diameter and SRL were nonsignificant (diameter: Holm-adjusted $p = 0.979$; SRL: Holm-adjusted $p = 0.661$; Table 7).

Table 7. Scenario-adjusted correlations between ECM colonization rate and root traits in Scots pine and Norway spruce seedlings. Partial Spearman rank correlations between the ECM colonization rate and root traits were calculated separately within each species after accounting for differences among the five experimental scenarios. Holm adjustment was applied jointly to the six correlations. Significant associations ($p < 0.05$) are shown in bold. ECM, ectomycorrhizal; SRL, specific root length; RTD, root tissue density.

Species	Root trait	Partial Spearman's r_s	Holm-adjusted p
Scots pine	Average root diameter	0.199	0.979

Species	Root trait	Partial Spearman's r_s	Holm-adjusted p
Norway spruce	SRL	−0.021	0.979
	RTD	−0.198	0.979
	Average root diameter	0.189	0.979
	SRL	0.256	0.661
	RTD	**−0.459**	**0.029**

4. Discussion and Conclusions

Under the treatment conditions and duration of this experiment, the accumulation and allocation of seedling biomass were primarily influenced by temperature, whilst water treatment had a clear effect on root functional traits and the ECM colonization rate.

4.1 Biomass and allocation

Our first hypothesis was only partially supported. Warming increased the total biomass of seedlings and reduced the root-to-shoot ratio, while the water treatment had no significant effect on these two parameters. Total biomass, needle biomass and stem biomass all increased with warming, and there was no evidence of significant differences in these responses between the two species. In contrast, root biomass exhibited a significant temperature × water condition × species interaction, indicating that its response depends on the specific species and water condition combination.

The growth response of northern tree species seedlings to warming is context-dependent and depends on tree species, treatment intensity and resource conditions. A synthesis of experimental studies found that elevated temperatures generally caused a more positive growth responses particularly for temperate and boreal species(Way & Oren 2010). Controlled field experiments in central Finland likewise showed that moderate warming increased the growth and biomass of Scots pine and Norway spruce seedlings (Nissinen et al. 2020; Pikkarainen et al. 2022). These findings are broadly consistent with the positive biomass response to warming observed in our experiment.

Our study did not measure photosynthesis or plant respiration. It is therefore not possible to determine the physiological mechanisms by which warming promotes biomass accumulation. Given that warming was limited to 4°C above ambient temperature and that the maximum temperature reached only 21°C, the treatment likely remained within or close to a favourable photosynthetic temperature. In mature Scots pine growing in northern Sweden, the optimum temperature of light-saturated net photosynthesis varied seasonally from approximately 17.5 to 23°C, with 20°C representing the season-long optimum (Stangl et al. 2022). An optimum of approximately 25°C has also been reported for light-saturated photosynthesis in Norway spruce saplings (Šigut et al. 2015). We cautiously speculate that warming may enhance photosynthesis in seedlings and improve the early growth of pine and spruce seedlings in our experiment.

Water conditions did not exhibit a consistent main effect on total biomass or most biomass components. This may be related to the intensity and duration of the drought treatment. Severe water limitation does indeed reduce the biomass of pine and spruce, but if the degree of water stress is mild, the differences in biomass may not be significant. Musella et al. (2025) found that a 22-day treatment of moderate water limitation (30~35 % field water holding capacity) did not significantly affect the growth parameters of Scots pine seedlings, although the treatment significantly reduced stomatal conductance and transpiration rates. Zlobin et al. (2019) similarly found that three-month-old Scots pine and Norway spruce seedlings exhibited overall strong stability in dry matter accumulation under water restriction lasting 40 days. Their 50% perlite water-holding capacity treatment did not significantly affect dry matter accumulation in either species. The substrate used in the two studies mentioned above was perlite, which has different water-retention properties compare with forest soil, the physiological drought severity of plants between our experiments can not directly compare. While based on the treatment targets reported in the respective methods, the ratio of water mass in drought-treated pots to that in control pots was 0.57 in our experiment, compared with 0.50 in Zlobin et al. (2019) and approximately 0.35 in Musella et al. (2025); In our experiment, the target water mass in drought-treated pots represented a larger proportion of that in control pots in our experiment than in the two comparison studies. Together, these results support the conclusion that the biomass of pine and spruce seedlings does not necessarily respond significantly to moderate or short-term water limitation.

Although there were no significant differences between the two species in their total biomass response to the interaction between warming and water condition, the root biomass exhibited a significant temperature × moisture × species interaction. Inspection of the treatment means suggested that warming was associated with a larger increase in root biomass under drought than under wet conditions in Norway spruce, whereas Scots pine showed the reverse numerical pattern. However, the root-to-shoot ratio showed only main effects of temperature and species, with all interactions being nonsignificant. The significant interaction in root biomass may represent an early belowground response to the combined treatments. Such responses may be diluted when expressed at the whole-plant level. This interpretation is consistent with controlled studies of Norway spruce seedlings and saplings showing that root and shoot growth respond differently to soil temperature and are not temporally synchronized (Lahti et al. 2005; Kilpeläinen et al. 2019). Field observations of Scots pine likewise showed that root growth occurred later than aboveground growth and that different root types differed in their sensitivity to summer drought (Ding et al. 2020). This suggests that the roots of seedlings may have responded to changes in moisture and

temperature, although their specific responses may be obscured by the plant's overall response.

4.2 Root traits

Our second hypothesis was generally supported. RTD was significantly influenced by temperature and moisture, and none of the interactions were significant. Under warming conditions, the recovery treatment reduced the RTD across species. In contrast, SRL exhibited a significant temperature × moisture × species interaction. In Scots pine, SRL was lower under ambient–drought treatment and higher under ambient-wet treatment; however, there were no significant differences in SRL amongst the three warm moisture-status treatments. In Norway spruce, SRL was significantly lower under the warmed–drought treatment than warmed–wet treatment. Recovery increased SRL in Norway spruce, resulting in an intermediate value that differed significantly from both the drought and wet treatments. Although the RTD of both species exhibited relatively consistent responses to temperature and moisture, SRL and its response to moisture condition in warmed groups showed a greater species-specific variation. Correlation analysis further indicated that SRL was significantly negatively correlated with RTD in both species.

Total root length and number of root tips exhibited similar responses to the treatments. Both metrics were significantly influenced by temperature, water condition and species, while all interactions were nonsignificant. Warming and wet conditions generally increased the total root length and root tip number of seedlings. Total root length and root tip number were higher under warmer and moister conditions, and this response pattern did not vary significantly between species; therefore, under the treatments set in our experiment, warming and wet conditions may favour the development of larger root systems in both species. This result is consistent with the findings of Domisch et al. (2001), who reported that higher soil temperatures promote root extension in Scots pine seedlings, and is also in line with the observations of Rikala and Puttonen (1988), who found that low temperatures combined with root water loss inhibit new root formation.

The discrepancy between the similar responses of the two species to RTD and their responses to SRL suggests that changes in environmental conditions do not necessarily cause root traits to respond in a single direction. In the case of Norway spruce, higher RTD and lower SRL under warming–drought conditions may indicate a root system with denser tissue and lower elongation capacity per unit mass; water recovery treatment increased SRL and brought RTD to a level close to that of the continuously moist treatment, suggesting that its root system traits may be relatively sensitive to changes in water conditions. In contrast, although

the RTD of Scots pine also responded to water status, there was no significant difference in its SRL across the three warming–moisture treatments. Similar species differences have also been observed in previous studies. Ostonen et al. (2007) found that the short roots of Norway spruce were more sensitive to site conditions than those of Scots pine. Meanwhile, long-term irrigation experiments on Scots pine indicated that increased water availability can significantly enhance fine-root biomass and root length, but does not necessarily result in changes in SRL, RTD or root diameter (Brunner et al. 2019).

At the individual level, SRL in both species was significantly negatively correlated with RTD, while RTD was also significantly negatively correlated with root diameter; the direction of these relationships is consistent with the study by Bergmann et al. (2020), which noted a strong negative correlation between SRL and root diameter, interpreting this relationship as a cooperative gradient characterized by a transition from a self-exploratory strategy involving fine roots and high SRL to one involving coarser roots and higher potential for mycorrhizal collaboration. In the present study, however, the negative correlation between SRL and average root diameter was significant only in Norway spruce; in Scots pine, the correlation did not reach the level of significance. Such species-specificity in trait correlation is not unexpected. Relationships among root diameter, SRL and RTD may vary with phylogenetic context, mycorrhizal association and the scale of observation (Kong et al. 2019; An et al. 2022). Evidence at the intraspecific level also indicates that these relationships are not fixed. In interior Douglas-fir, RTD was negatively correlated with root diameter, whereas the diameter–SRL relationship was weak, and more than half of the variation in morphological traits occurred at the root-branch and soil-block scales (Defrenne et al. 2019); similarly, across Japanese cedar provenances grown in a common garden, SRL decreased with root diameter, whereas RTD showed no significant relationship with diameter (Nakahata et al. 2024).The absence of a significant SRL–diameter relationship in Scots pine may therefore reflect a more restricted variation in root diameter or a different relative contribution of diameter and tissue density to SRL variation.

It should also be noted that the three traits are not mathematically independent. In our experiment, both root length and root diameter were obtained by analyzing the scanned root images using the RhizoVision software; SRL was calculated directly from root length and root dry mass, whilst RTD was calculated from root volume and root dry mass as provided by RhizoVision. When RhizoVision approximates the root system as a cylinder, both root volume and total length are calculated based on length between the skeletal pixel (The basic units for analyzing the root system images) (Seethepalli et al. 2021). Consequently, the

correlations mentioned above may also stem in part from the mathematical relationships arising from the definition of these traits and the estimation of root volume.

Besides, as root dry mass occurs in the denominator of SRL and the numerator of RTD, variation in root dry mass could theoretically contribute to a negative correlation between these two traits. To test whether root dry mass was the main driver of this relationship, we examined its correlations with SRL and RTD separately for each species. If root dry mass alone dominated these ratios, the expected pattern would be a negative correlation between root dry mass and SRL and a positive correlation between root dry mass and RTD. However, this pattern was not observed. In Norway spruce, root dry mass was almost unrelated to SRL and was not significantly related to RTD. In Scots pine, the correlation coefficient was positive for the relationship between root dry mass and SRL and negative for the relationship between root dry mass and RTD. Although these results do not rule out the potential impact of mathematical coupling, they suggest that variation in root biomass may not be the main cause of the observed negative SRL–RTD correlations.

4.3 Colonization rate

Our third hypothesis was partially supported. ECM colonization responded to moisture conditions, but the direction and magnitude of this response depended strongly on species and temperature context.

For Norway spruce, mean ECM colonization was lowest in the warmed–drought treatment and was significantly lower than in the ambient–drought, ambient–wet, and warmed–wet treatments. The ECM colonization did not differ significantly between the ambient-wet and warmed-wet treatment groups.

Previous studies have shown that prolonged or severe water limitation can reduce ECM development in conifer roots. In Norway spruce, drought reduced parent-root elongation and the number of growing mycorrhizal root tips (Feil et al. 1988); longer-term experiments reported significant reductions in overall mycorrhizal colonization and active mycorrhizal density (Nilsen et al. 1998; PeÅ¡kovÃ¡ et al. 2015; Nickel et al. 2017). Similar responses have also been observed in other conifers: extreme water stress reduced the root colonization of *Pinus tabulaeformis* and *Pinus sylvestris* (Wang et al. 2021b; Musella et al. 2025). These reductions may be caused by restricted root elongation and the reduced availability or survival of suitable root tips, or by the direct inhibition of ECM fungal growth under low water availability (Feil et al. 1988; Coleman et al. 1989). Although this experiment did not investigate the specific mechanism of the

decline in mycorrhizal colonization rates, these studies provide some evidence to partially explain the particularly low ECM colonization rates observed in Norway spruce under warmed–drought treatment group.

Under warming condition, ECM colonization in Norway spruce was higher after recovery than under continued drought but remained significantly lower than under wet conditions. As our study only measured ECM colonization rates at the end of the experiment and did not test the dynamic recovery process following rewatering, the final colonization rate results allow us only to speculate that water recovery treatment may be conducive to the re-establishment of ECM in spruce. Several studies involving dynamic observations support this interpretation. Möttönen et al. (2005) compared root conditions of spruce seedlings at the end of the drought period and 8 days after rewatering, finding that differences in colonization ratio between the drought treatment and the control had diminished or were no longer significant following rewatering. In a longer-term field experiment with repeated sampling after rewatering, Danzberger et al. (2023) found that the degree of mycorrhization in previously drought-exposed Norway spruce initially declined after rewatering, began to increase from approximately day 28, and no longer differed significantly from the control by day 42.

Scots pine exhibited a different response pattern from Norway spruce, with its ECM colonization rate remaining at a relatively high level across all five scenarios. Colonization rates did not differ significantly between the ambient–drought and warmed–recovery groups, and both were significantly lower than in the other three treatment groups.

It is worth noting that the ECM colonization rate under the warmed–recovery treatment was significantly lower than that under the warmed–drought and warmed-wet treatments. One possible explanation is that the ECM colonization rate is defined as the proportion of colonized root tips out of the total number of root tips. If rewatering promotes the formation of new roots and root tips, and these new root tips have not yet been colonized by ECM fungi, then the colonization rate may temporarily decline. In this experiment, the mean total root length and number of root tips in the recovery treatment were both higher than those in the sustained drought treatment, which is consistent with this explanation in terms of direction. As we lack more detailed data, this mechanism is still speculative at present, and more evidence of the dynamics of mycorrhizal colonization is needed to confirm it. Li et al. (2015) proposed a similar explanation: in their study, warming increased the fine root biomass of *Picea asperata* seedlings while simultaneously reducing the ECM colonization rate. The

authors hypothesized that increased root growth might reduce the overall colonization rate by increasing the proportion of non-mycorrhizal roots.

Correlation analysis further indicated that average root diameter was not significantly correlated with ECM colonization rate in either species. Consequently, when using the ECM colonization rate of root tips as an indicator of mycorrhizal establishment, our experiment found no evidence that the colonization rate increased with an increase in average root diameter or a decrease in SRL. This differs from the trait coordination relationships expected under the 'do-it-yourself–outsourcing' collaboration gradient proposed by Bergmann et al. (2020).

This inconsistency requires careful interpretation. The framework developed by Bergmann et al. (2020) is primarily based on comparisons of average traits across a large number of plant species; the colonization data used were derived mainly from plants associated with arbuscular mycorrhizal (AM) fungi and the indicator employed was the proportion of colonized root length rather than the proportion of ECM-colonized root tips. The biological implications and measurement bases of these two indicators are not entirely the same. In the discussion by Bergmann et al. (2020), thicker roots typically possess greater cortical space, thereby providing mycorrhizal fungi with more intra-root habitat. ECM fungi, however, form mycelial sheaths on the root tip surface and establish interfaces for resource exchange via the intercellular Hartig net. The importance of cortical area for ECM symbiosis cannot be ruled out, while the promotional effect of increased root cortical space on AM symbiosis may be more direct (Yan et al. 2022). Besides, a larger cortical space may influence the symbiotic relationship within an individual colonized root tip, rather than increasing the probability of root tip colonization. Furthermore, other studies, like Comas et al. (2014) found that ECM formation was independent of root diameter and SRL, while Matthus et al. (2025) found no general evidence linking root diameter to ECM colonization; therefore, the suitability of average root diameter as a surrogate marker for ECM root-tip colonization may be very limited.

At the same time, the rate of ECM colonization at the root tips does not directly quantify the functional contribution or the efficiency of resource exchange. The resource acquisition capacity of ECM fungi also depends on the biomass, mycelial extent, fungal community composition and physiological activity of extra-radical hyphae. Previous studies have indicated that information on ECM colonization at the root tip and community composition can only reflect the distribution and function of extra-radical hyphae in the soil to a limited extent (Anderson & Cairney 2007). Therefore, the variations in colonization rates

observed in this study may not fully reflect changes in the symbiotic relationship between seedlings and fungi, and further in-depth research is required.

4.4 Similar biomass may mask belowground changes

Under ambient temperature, wet Scots pine seedlings had significantly higher SRL and ECM colonization than drought-exposed seedlings. Similarly, under warming, drought-exposed Norway spruce seedlings had significantly lower SRL and ECM colonization than wet seedlings. Unlike the species-specific responses of SRL and ECM colonization, RTD showed a consistent response across species. Pooled-species comparisons showed that drought significantly increased the RTD of seedlings; RTD in warmed-recovery seedlings was lower than that in warmed-drought seedlings and did not differ from that in warmed-wet seedlings. Moisture availability was reflected in root length deployment, tissue construction, and ECM colonization. The SRL and ECM responses were species-specific, whereas the RTD response was shared across species. Thus, sufficient water availability was associated with greater root length per unit biomass and greater ECM colonization, whereas drought reduced both components of belowground resource acquisition.

This experient observed that root exploration and ECM association did not function as simple alternative pathways. Environmental conditions may simultaneously promote or constrain root-based and fungal-associated pathways of resource acquisition. Lulu et al. (2024) reported a similar pattern in *Quercus mongolica* and *Tilia amurensis*: extreme drought reduced both seedling SRL and the relative abundance of medium- and long-distance ECM exploration types. Under the conditions of their experiment, ECM exploration declined alongside root exploration rather than compensating for it.

Furthermore, these belowground changes occurred without a detectable moisture effect on total biomass. Biomass integrates growth over time. In our experiment, changes in root traits and ECM colonization may become detectable before the climate consequences are reflected in whole-plant biomass. These changes may represent an early belowground indication of drought stress. Further measurements of nutrient acquisition, growth and survival would be required to determine their functional consequences.

Taken together, for seedlings in boreal forests, assessments based solely on seedling size or biomass may overlook early changes in belowground resource-acquisition structures. Root functional traits and ECM colonization may therefore provide complementary indicators of seedling responses to changing moisture

conditions, particularly when aboveground or whole-plant growth responses have not yet become apparent.

4.5 Conclusion

In this experiment, we found that warming increased total, needle and stem biomass and reduced root-to-shoot ratio in both pine and spruce seedlings, while water treatment had showed significant effect on most biomass components. However, water treatment affected several belowground characteristics, including total root length, number of root tips, average root diameter, SRL, RTD and ECM colonization. Similar biomass outcomes may mask variation in root morphology, tissue construction and symbiotic status.

Some of the belowground responses were strongly species-specific. Under warmed conditions, pine maintained a high colonization rate under both water treatments; for spruce, low water availability significantly reduced the colonization rate under both temperature conditions. When water availability was low, higher temperatures increased the SRL in pine, but this trend was not observed in spruce seedlings. Across individual seedlings, SRL and RTD were negatively correlated in both species. This lends partial support to the root economics spectrum hypothesis.

In the context of gradual global warming and an increasing risk of drought, our findings indicate that short-term warming can promote seedling biomass, which may not accompanied by detectably reduced under moderate water limitation. Nevertheless, root traits and ECM colonization may had already responded to the water treatments. How these changes observed below ground affect the seedling stress tolerance and long-term growth still remain unknow and long term experiments are needed for assessment.

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Popular science summary

Boreal forests are facing rising temperatures and more frequent droughts due to climate change. Young tree seedlings, such as pine or spruce, with their small root systems and limited energy reserves, may be particularly vulnerable to these changes. In this study, Scots pine and Norway spruce seedlings were cultivated in climate chambers, simulating current temperatures and a 4 °C temperature increase, respectively. Treatments involving ample water, drought and post-drought rewatering were established to understand how the seedlings respond to changes in temperature and water availability.

After three and a half months of cultivation, the warmer conditions increased the seedlings' total biomass and reduced the relative proportion allocated to the roots. Water conditions did not significantly alter the seedlings' total biomass. However, drought did alter the growth of the seedlings' underground parts. Under drought conditions, the root tissue of the seedlings became denser, and roots of the same weight were shorter in length. Under warming conditions, drought significantly reduced the colonization rate of symbiotic mycorrhizal fungi on the root tips of Norway spruce, although Scots pine generally maintained a high level of colonization. Following re-watering, some root characteristics and mycorrhizal colonization in Norway spruce recovered to levels close to those of seedlings that had been continuously watered.

These results suggest that even when the total biomass of seedlings does not exhibit a clear response to drought, changes may still have occurred in their root systems and mycorrhizal partners. Longer-term observations will be required to determine how these changes will ultimately affect the growth and survival of the seedlings.

Appendix

Climate-chamber settings

Daily maximum and minimum air temperatures from 1st June to 15th September in 2022–2024 were aligned by day of the growing-season period and averaged across the three years. Second-order polynomial models were then fitted separately to mean daily maximum and minimum.Daily photoperiod was calculated for the latitude of the study site (64.25° N) from solar declination and day of year. For each day, a 24 h cosine curve spanning the fitted minimum and maximum temperatures was generated. The mean of the warmest hourly values, where was the rounded photoperiod in hours, was used as the light-period temperature, and the mean of the remaining hourly values was used as the dark-period temperature. Daily values were subsequently averaged within seven-day intervals and rounded to obtain the weekly chamber settings presented in Table A1.

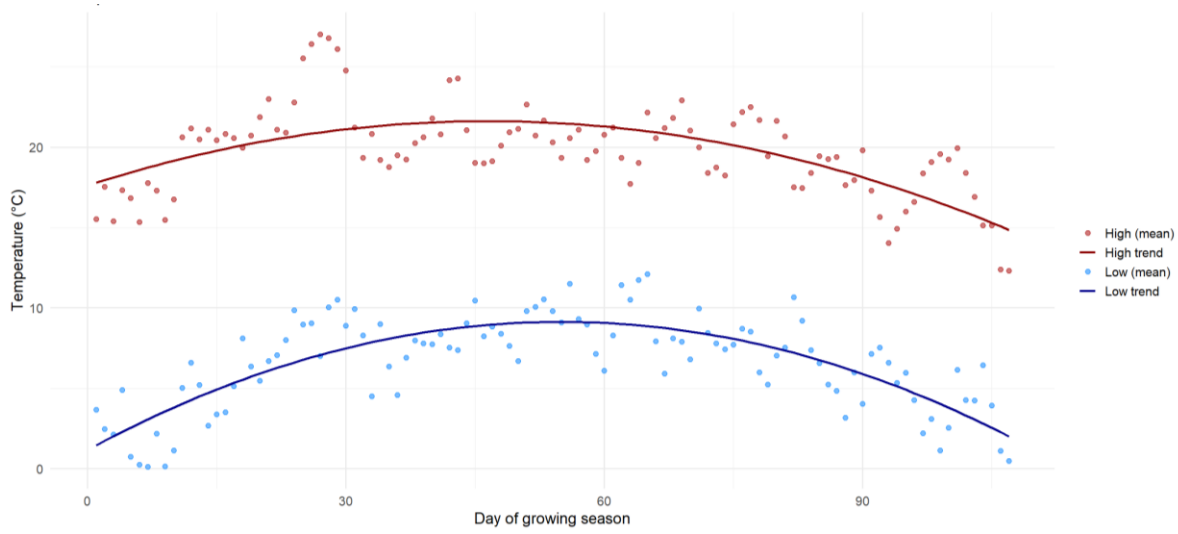


Figure A1. Three-year mean daily maximum and minimum air temperatures used to derive the ambient climate-chamber temperature schedule. Points show daily maximum and minimum temperatures averaged across 2022–2024 for each day from 1st June to 15th September, based on meteorological records from the Åheden AWS. Solid red and blue lines represent second-order polynomial fits to the mean daily maximum and minimum temperatures, respectively.

Table A1. Weekly environmental settings used in the climate-chamber experiment. Dates indicate when each set of environmental conditions was initiated. The warmed treatment was generally maintained at approximately 4 °C above the ambient treatment. Temperature treatments were alternated between climate chambers KK5 and KK6 during the experiment. Relative humidity was maintained at 90%, and photosynthetically active radiation (PAR) was maintained at 200 µmol m^{-2} s^{-1} throughout the experiment.

Setting date	Week	photoperiod (h d^{-1})	Ambient chamber	Ambient day/night (°C)	Warmed chamber	Warmed day/night (°C)
2026-01-05	1	20	KK5	12/5	KK6	16/9
2026-01-12	2	20	KK6	13/5	KK5	17/9
2026-01-19	3	21	KK5	14/6	KK6	18/10
2026-01-26	4	20	KK6	15/7	KK5	19/11
2026-02-02	5	20	KK5	16/8	KK6	20/12
2026-02-09	6	20	KK6	16/9	KK5	20/13
2026-02-16	7	19	KK5	17/9	KK6	21/13
2026-02-23	8	19	KK6	17/10	KK5	21/14
2026-03-02	9	18	KK5	17/10	KK6	21/14
2026-03-09	10	17	KK6	17/10	KK5	21/14
2026-03-16	11	16	KK5	17/9	KK6	21/13
2026-03-23	12	16	KK6	16/8	KK5	20/12
2026-03-30	13	15	KK5	15/7	KK6	19/11
2026-04-07	14	14	KK6	14/7	KK5	18/11
2026-04-15	15	13	KK5	13/5	KK6	17/9
2026-04-23	16	13	KK6	12/4	KK5	16/8

Analysis of experimental scenarios

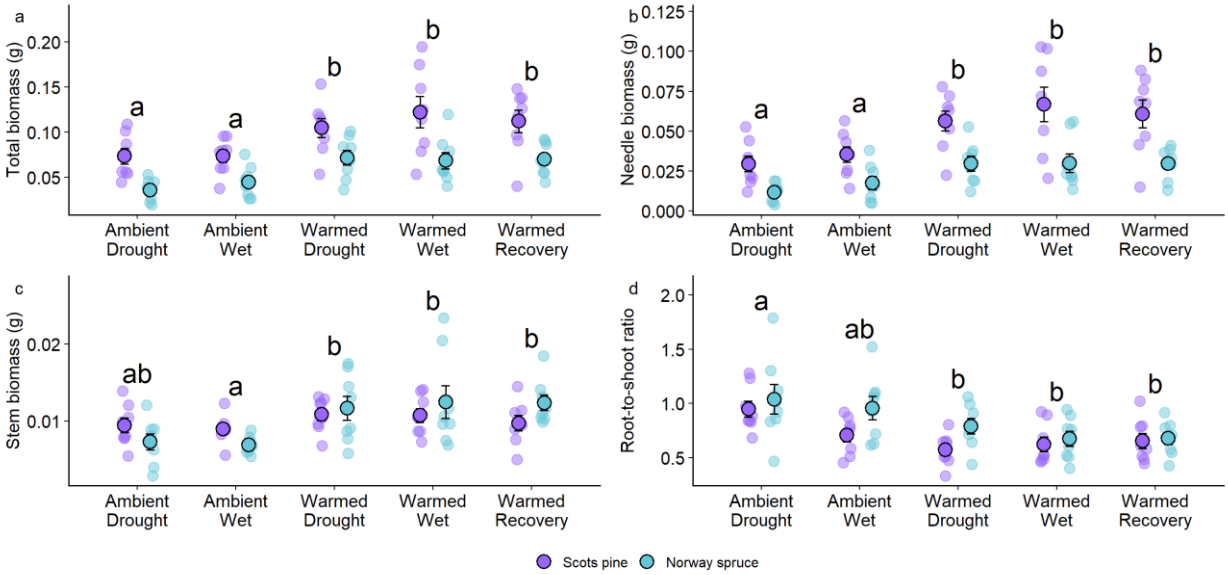


Figure A2. Total biomass (a), needle biomass (b), stem biomass (c), and root-to-shoot ratio (d) of Scots pine and Norway spruce seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Different lowercase letters indicate significant differences among scenarios averaged across species (Holm-adjusted $p < 0.05$).



Figure A3. Stem biomass of Scots pine (left) and Norway spruce (right) seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly within each species (Holm-adjusted $p < 0.05$).

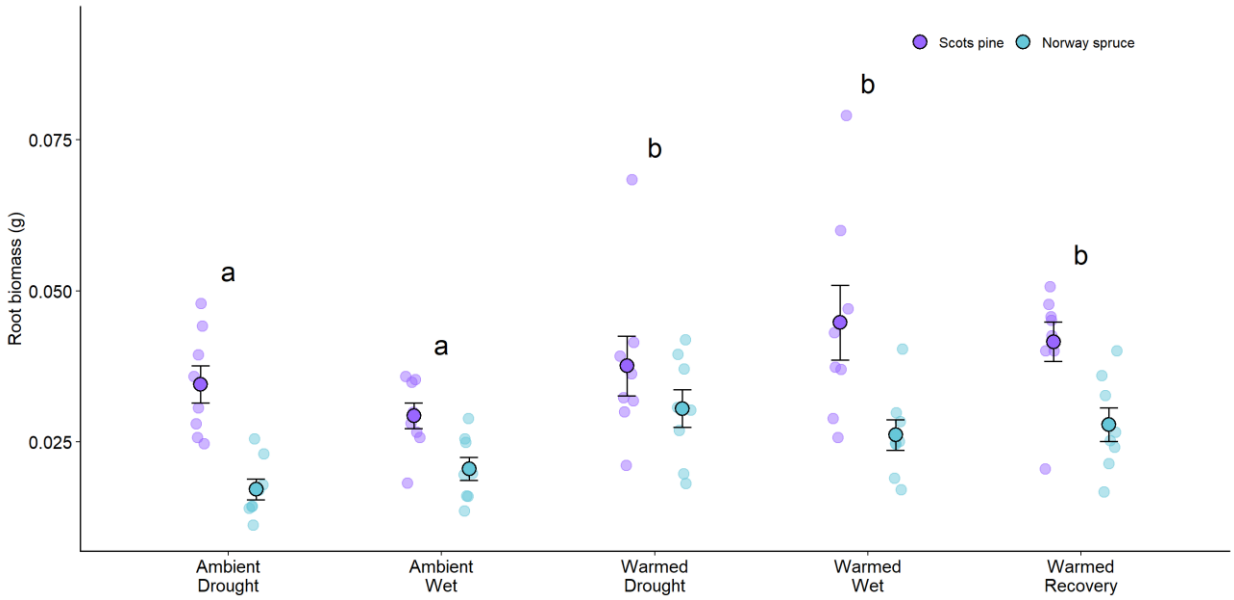


Figure A4. Root biomass of Scots pine and Norway spruce seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly based on estimated marginal means averaged across species (Holm-adjusted $p < 0.05$).

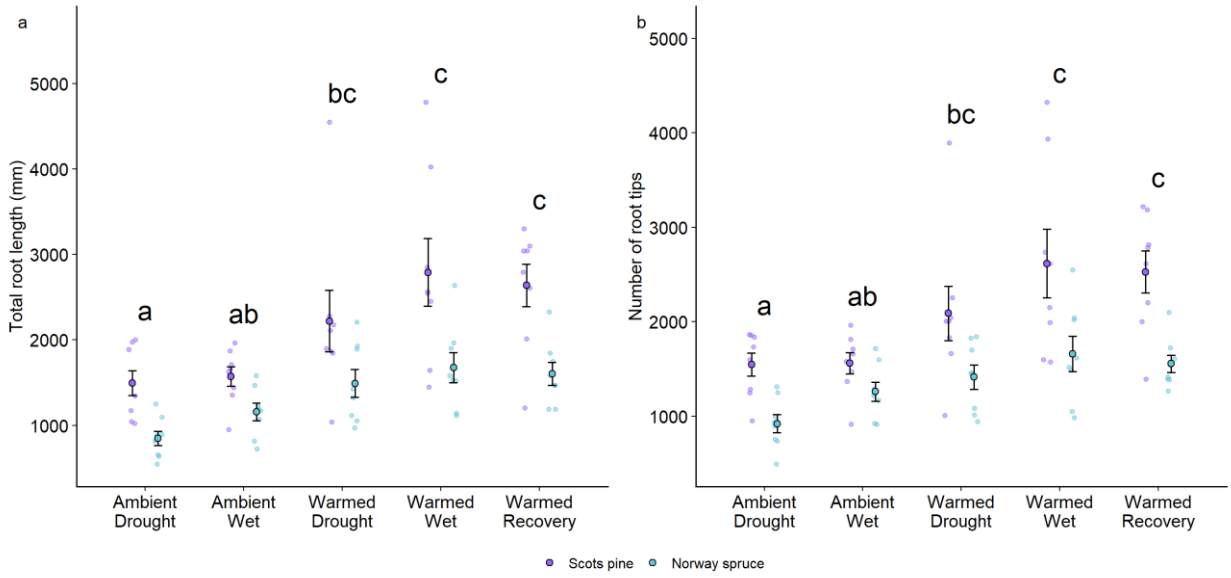


Figure A5. Total root length (a) and number of root tips (b) of Scots pine and Norway spruce seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly based on estimated marginal means averaged across species (Holm-adjusted $p < 0.05$).

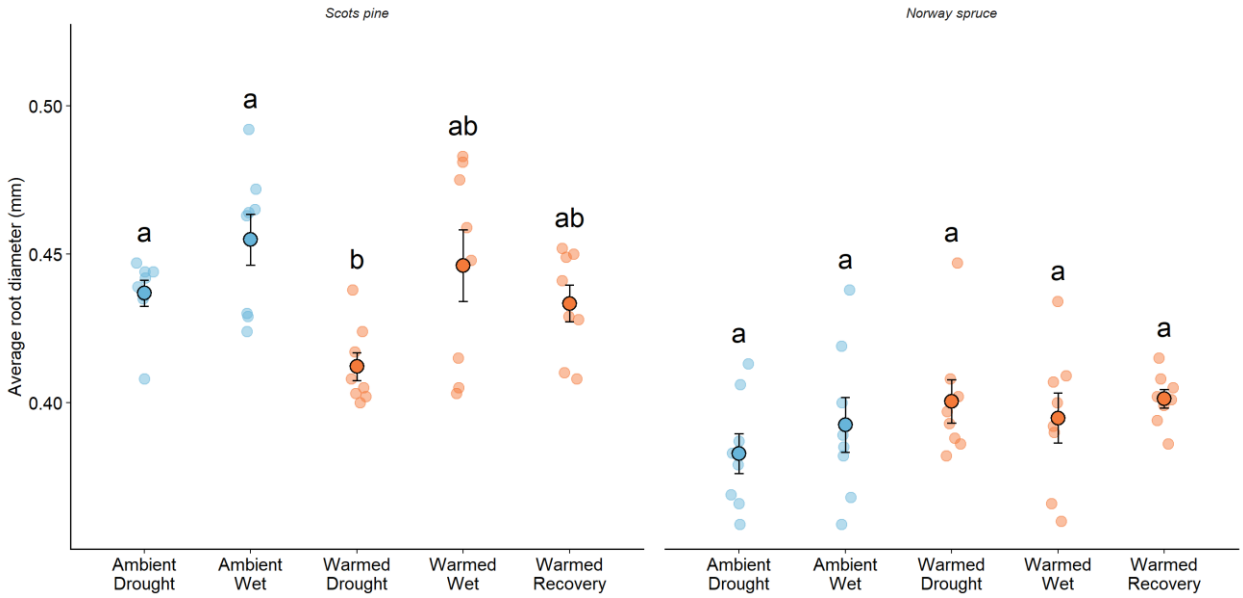


Figure A6. Average root diameter of Scots pine (left) and Norway spruce (right) seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly within each species (Holm-adjusted $p < 0.05$).

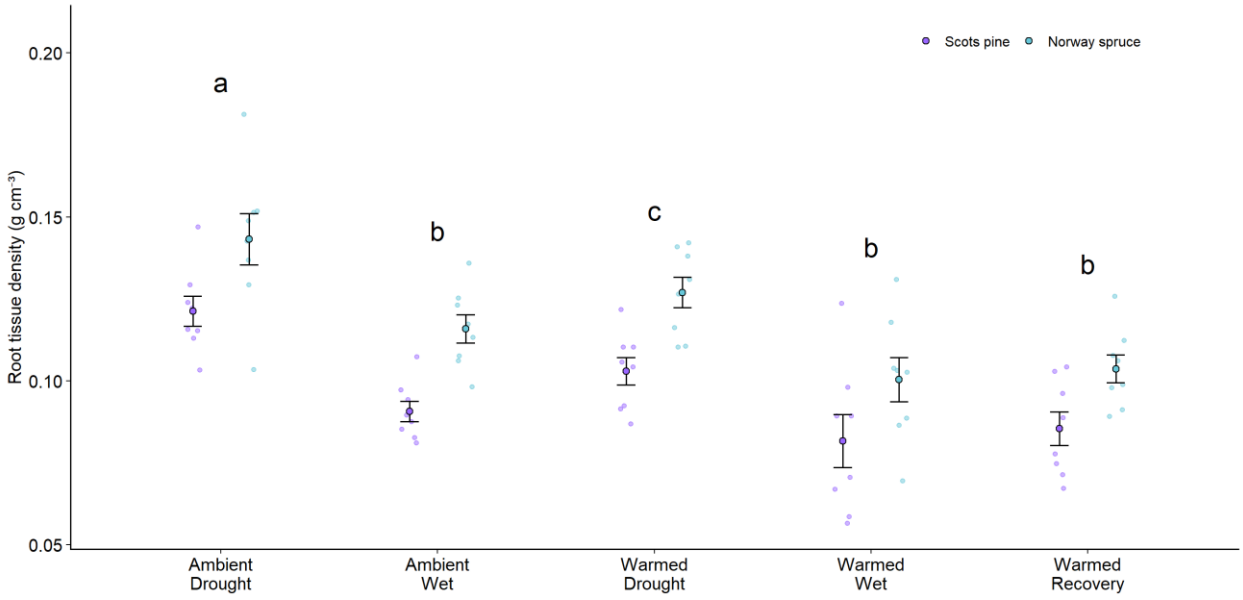


Figure A7. Root tissue density (RTD) of Scots pine and Norway spruce seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly based on estimated marginal means averaged across species (Holm-adjusted $p < 0.05$).

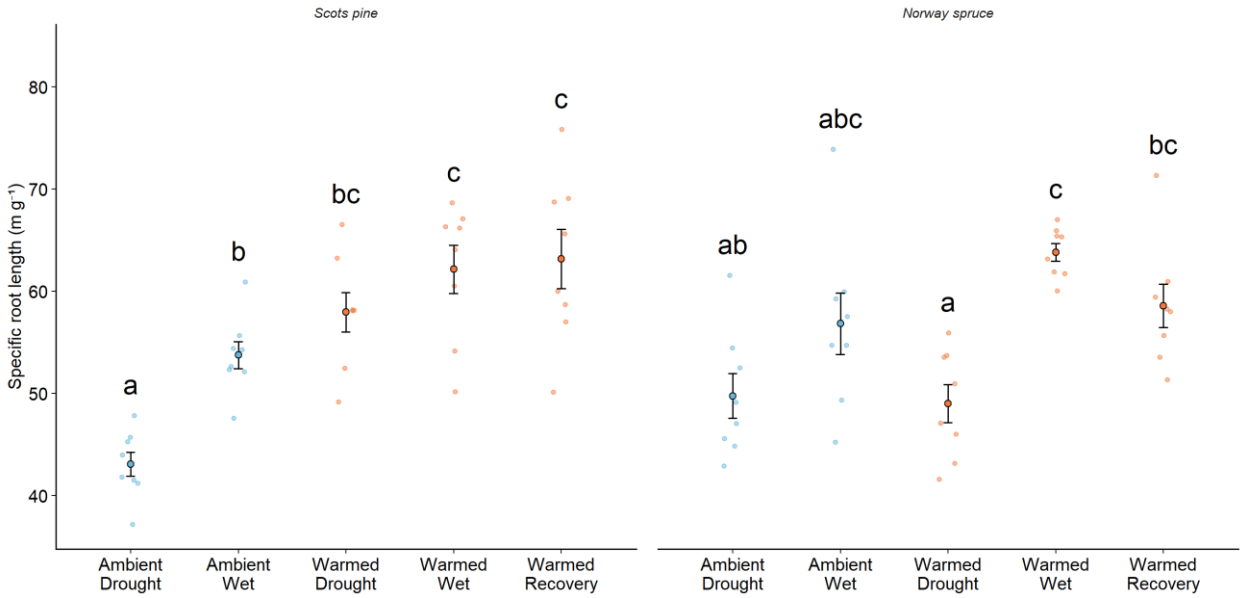


Figure A8. Specific root length (SRL) of Scots pine (left) and Norway spruce (right) seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly within each species (Holm-adjusted $p < 0.05$).

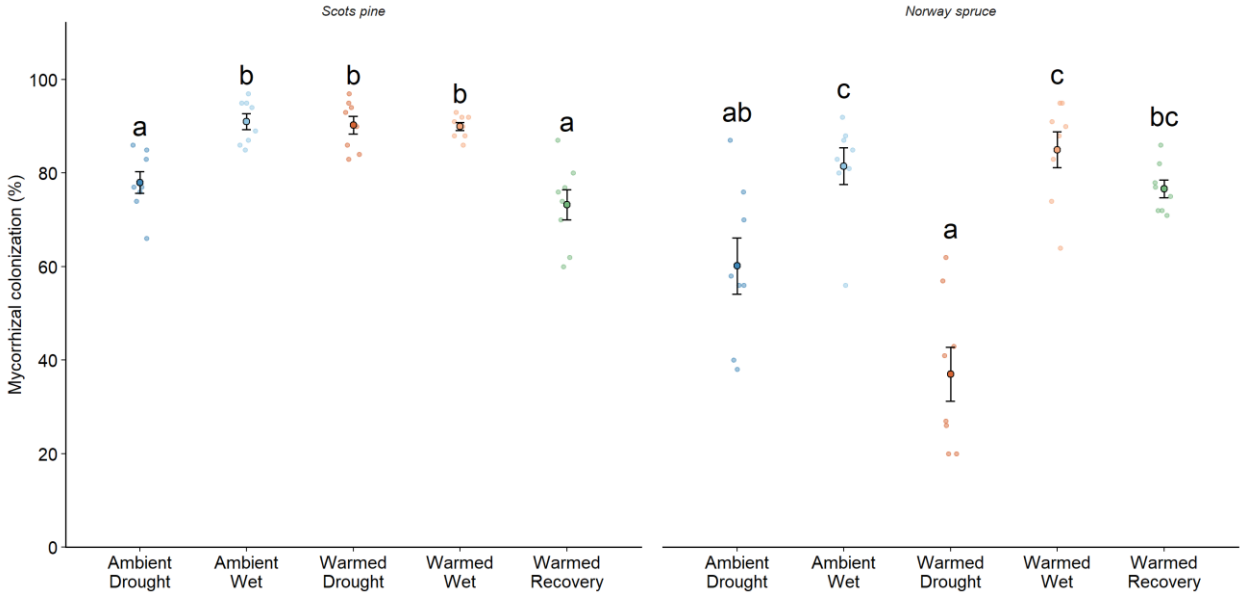


Figure A9. Ectomycorrhizal colonization (%) of Scots pine (left) and Norway spruce (right) seedlings across five experimental scenarios. Small points represent individual seedlings; large points and error bars indicate means ± SE. Scenarios sharing no lowercase letters differ significantly within each species (Holm-adjusted $p < 0.05$).

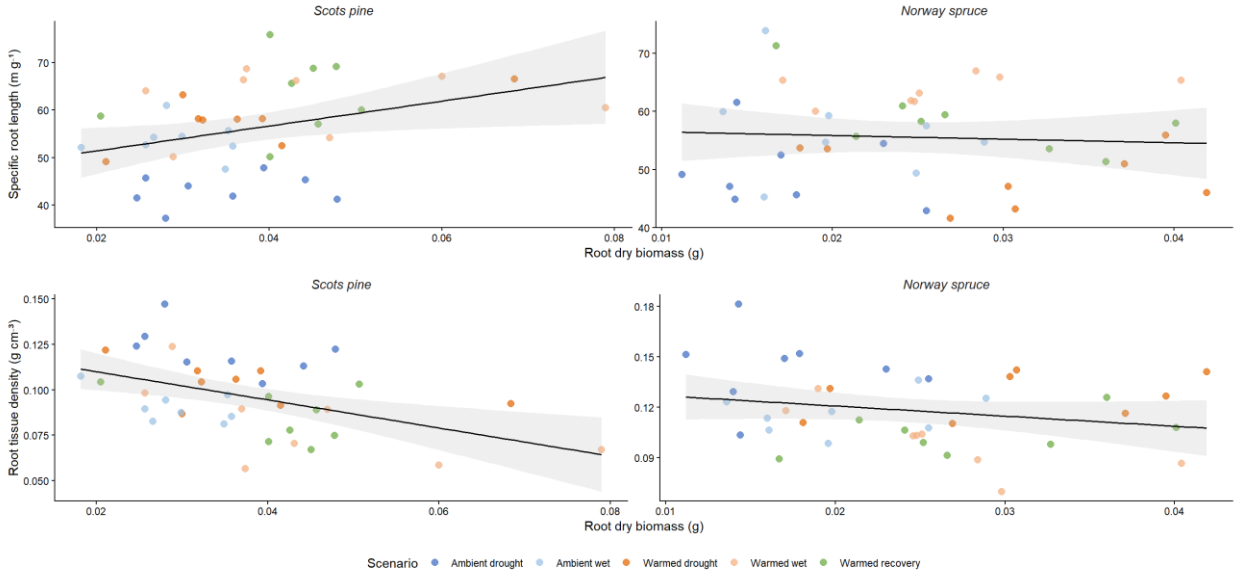


Figure A10. Relationships between root dry biomass and specific root length (SRL; top row) and root tissue density (RTD; bottom row) in Scots pine (left) and Norway spruce (right). Points represent individual seedlings, with colours indicating experimental scenarios. Black lines show linear fits to untransformed data pooled across scenarios within each species; shaded bands indicate 95% confidence intervals.

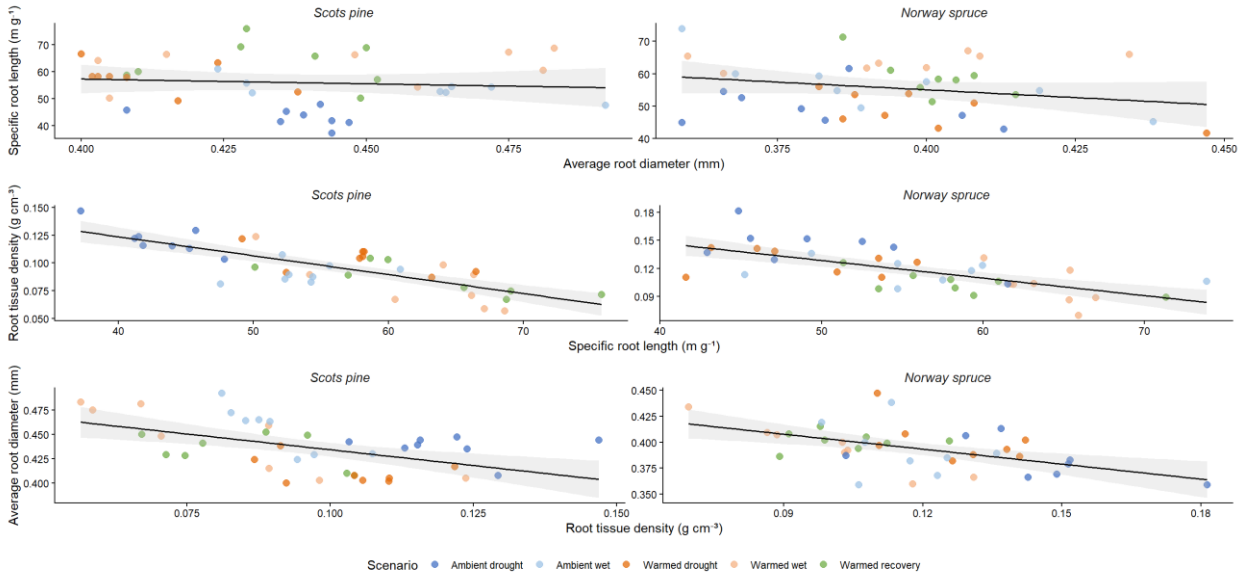


Figure A11. Pairwise relationships among average root diameter, specific root length (SRL), and root tissue density (RTD) in Scots pine (left) and Norway spruce (right). Points represent individual seedlings, with colours indicating experimental scenarios. Black lines show linear fits to untransformed data pooled across scenarios within each species; shaded bands indicate 95% confidence intervals.

Detailed results of post hoc comparisons and correlation analyses

Table A2. Post hoc comparisons of root tissue density among water-status treatments under warmed conditions (Analysis 2). Comparisons are based on estimated marginal means averaged across species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the three comparisons.

Contrast	Estimate (g cm^{-3})	SE	df	t	Holm-adjusted *p*
Drought − Wet	0.02391	0.00655	42	3.651	0.0014
Drought − Recovery	0.02040	0.00490	42	4.162	0.0005
Wet − Recovery	−0.00351	0.00666	42	−0.526	0.6014

Table A3. Post hoc comparisons of specific root length among four temperature–water treatment combinations within each species (Analysis 1). AD, ambient–drought; AW, ambient–wet; WD, warmed–drought; WW, warmed–wet. Comparisons are based on estimated marginal means within each species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the ten comparisons within each species.

Species	Contrast	Estimate (m g^{-1})	SE	df	t	Holm-adjusted *p*
Scots pine	AD − WD	−14.8815	2.4151	56	−6.162	4.139×10^{-7}
Scots pine	AD − AW	−10.6710	1.9009	56	−5.614	2.562×10^{-6}
Scots pine	AD − WW	−19.0682	2.8237	56	−6.753	5.313×10^{-8}
Scots pine	WD − AW	4.2105	2.5075	56	1.679	0.1974
Scots pine	WD − WW	−4.1867	3.2631	56	−1.283	0.2048
Scots pine	AW − WW	−8.3972	2.9032	56	−2.892	0.0163
Norway spruce	AD − WD	0.7561	3.0655	56	0.247	0.8061
Norway spruce	AD − AW	−7.0735	3.9633	56	−1.785	0.1663
Norway spruce	AD − WW	−14.0449	2.5002	56	−5.617	3.157×10^{-6}

Species	Contrast	Estimate (m g^{-1})	SE	df	t	Holm-adjusted *p*
Norway spruce	WD − AW	−7.8295	3.7840	56	−2.069	0.1663
Norway spruce	WD − WW	−14.8009	2.2049	56	−6.713	6.187×10^{-8}
Norway spruce	AW − WW	−6.9714	3.3425	56	−2.086	0.1663

Table A4. Post hoc comparisons of specific root length among water-status treatments under warmed conditions within each species (Analysis 2). Comparisons are based on estimated marginal means within each species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the three comparisons within each species.

Species	Contrast	Estimate (m g^{-1})	SE	df	t	Holm-adjusted *p*
Scots pine	Drought − Wet	−4.1867	3.2631	42	−1.283	0.5144
Scots pine	Drought − Recovery	−5.1796	3.7229	42	−1.391	0.5144
Scots pine	Wet − Recovery	−0.9929	4.0001	42	−0.248	0.8052
Norway spruce	Drought − Wet	−14.8009	2.2049	42	−6.713	1.125×10^{-7}
Norway spruce	Drought − Recovery	−9.5634	3.0369	42	−3.149	0.0060
Norway spruce	Wet − Recovery	5.2375	2.4650	42	2.125	0.0395

Table A5. Post hoc comparisons of ectomycorrhizal colonization among four temperature–water treatment combinations within each species (Analysis 1). AD, ambient–drought; AW, ambient–wet; WD, warmed–drought; WW, warmed–wet. Comparisons are based on estimated marginal means within each species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the six comparisons within each species.

Species	Contrast	Estimate	SE	df	t	Holm-adjusted p
Scots pine	AD − WD	−1.08	0.294	56	−3.65	0.00230

Species	Contrast	Estimate	SE	df	t	Holm-adjusted p
Scots pine	AD − AW	−1.16	0.286	56	−4.03	0.000841
Scots pine	AD − WW	−0.921	0.178	56	−5.17	1.94×10^{-5}
Scots pine	WD − AW	−0.0804	0.355	56	−0.226	1.000
Scots pine	WD − WW	0.154	0.275	56	0.560	1.000
Scots pine	AW − WW	0.234	0.267	56	0.879	1.000
Norway spruce	AD − WD	1.06	0.409	56	2.60	0.0238
Norway spruce	AD − AW	−1.12	0.387	56	−2.88	0.0169
Norway spruce	AD − WW	−1.47	0.440	56	−3.35	0.00581
Norway spruce	WD − AW	−2.18	0.367	56	−5.93	9.71×10^{-7}
Norway spruce	WD − WW	−2.54	0.422	56	−6.01	8.79×10^{-7}
Norway spruce	AW − WW	−0.358	0.402	56	−0.892	0.376

Table A6. Post hoc comparisons of ectomycorrhizal colonization among water-status treatments under warmed conditions within each species (Analysis 2). Comparisons are based on estimated marginal means within each species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the three comparisons within each species.

Species	Contrast	Estimate	SE	df	t	Holm-adjusted *p*
Scots pine	Drought − Wet	0.154	0.275	42	0.560	0.579
Scots pine	Drought − Recovery	1.32	0.314	42	4.21	0.000266
Scots pine	Wet − Recovery	1.17	0.210	42	5.58	4.80×10^{-6}

Species	Contrast	Estimate	SE	df	t	Holm-adjusted *p*
Norway spruce	Drought − Wet	−2.54	0.422	42	−6.01	1.15×10^{-6}
Norway spruce	Drought − Recovery	−1.80	0.301	42	−5.98	1.15×10^{-6}
Norway spruce	Wet − Recovery	0.739	0.342	42	2.16	0.0365

Table A7. Spearman correlations between ectomycorrhizal colonization and selected root traits within each species, pooled across the five experimental scenarios. SRL: specific root length; RTD: root tissue density. Correlations were calculated within each species without controlling for experimental scenario. p values were adjusted using the Holm method across all six comparisons.

Species	Root trait	n	Spearman's r_s	Unadjusted *p*	Holm-adjusted *p*
Scots pine	Average root diameter	40	0.102	0.533	1.000
Scots pine	SRL	40	0.122	0.452	1.000
Scots pine	RTD	40	−0.248	0.123	0.490
Norway spruce	Average root diameter	40	0.132	0.417	1.000
Norway spruce	SRL	40	0.637	9.75×10^{-6}	5.85×10^{-5}
Norway spruce	RTD	40	−0.625	1.64×10^{-5}	8.22×10^{-5}

Table A8. Spearman correlations between diameter, SRL, RTD and Root biomass. SRL: specific root length; RTD: root tissue density. Correlations were calculated within each species without controlling for experimental scenario. p values were adjusted using the Holm method across all six comparisons. Bold values indicate Holm-adjusted $p < 0.05$.

Species	Trait pair	n	Spearman's r_s	Unadjusted *p*	Holm-adjusted *p*
Scots pine	Diameter–SRL	40	−0.138	0.396	0.937
Scots pine	SRL–RTD	40	−0.715	2.22×10^{-7}	2.22×10^{-6}
Scots pine	RTD–Diameter	40	−0.536	0.000363	0.00290
Scots pine	Root biomass–SRL	40	0.376	0.0169	0.0846
Scots pine	Root biomass–RTD	40	−0.465	0.00251	0.0151
Norway spruce	Diameter–SRL	40	−0.164	0.312	0.937
Norway spruce	SRL–RTD	40	−0.700	4.93×10^{-7}	4.44×10^{-6}
Norway spruce	RTD–Diameter	40	−0.520	0.000589	0.00412
Norway spruce	Root biomass–SRL	40	−0.046	0.776	0.937
Norway spruce	Root biomass–RTD	40	−0.212	0.189	0.755

Table A9. Holm-adjusted p values for pairwise comparisons among the five experimental scenarios, averaged across species. AD, ambient–drought; AW, ambient–wet; WD, warmed–drought; WW, warmed–wet; WR, warmed–recovery. Comparisons are based on estimated marginal means averaged across species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the ten comparisons separately for each response variable.

Contrast	Total biomass	Needle biomass	Stem biomass	Root-to-shoot ratio	Root biomass	Total root length	Number of root tips	Root tissue density
AD − AW	1.0000	0.7484	1.0000	0.4672	1.0000	0.2539	0.4136	1.78×10^{-5}
AD − WD	0.0011	0.0003	0.1230	0.0092	0.0370	0.0022	0.0188	0.0228
AD − WW	0.0012	0.0016	0.1230	0.0046	0.0370	1.09×10^{-5}	0.0003	4.39×10^{-6}
AD − WR	0.0010	0.0005	0.1230	0.0067	0.0204	3.29×10^{-6}	1.09×10^{-5}	2.80×10^{-7}
AW − WD	0.0123	0.0193	0.0155	0.3408	0.0370	0.1277	0.4136	0.0384
AW − WW	0.0123	0.0199	0.0158	0.2219	0.0370	0.0016	0.0136	0.1673
AW − WR	0.0113	0.0193	0.0056	0.3153	0.0205	0.0009	0.0013	0.1673
WD − WW	1.0000	1.0000	1.0000	1.0000	1.0000	0.5610	0.4136	0.003
WD − WR	1.0000	1.0000	1.0000	1.0000	1.0000	0.5610	0.4136	0.0006
WW − WR	1.0000	1.0000	1.0000	1.0000	1.0000	0.8060	0.8833	0.6003

Table A10. Holm-adjusted p values for pairwise comparisons among the five experimental scenarios within each species. AD, ambient–drought; AW, ambient–wet; WD, warmed–drought; WW, warmed–wet; WR, warmed–recovery. SRL, specific root length; ECM, ectomycorrhizal. Comparisons are based on estimated marginal means within each species, using an HC3 robust covariance matrix. p values were adjusted using the Holm method across the ten comparisons separately for each species and response variable.

Species	Contrast	Stem biomass	Average root diameter	SRL	ECM colonization
Scots pine	AD − AW	1.0000	0.4304	2.60×10^{-6}	0.0008

Species	Contrast	Stem biomass	Average root diameter	SRL	ECM colonization
Scots pine	AD − WD	1.0000	0.0052	3.64×10^{-7}	0.0025
Scots pine	AD − WW	1.0000	1.0000	3.51×10^{-8}	1.90×10^{-5}
Scots pine	AD − WR	1.0000	1.0000	6.31×10^{-7}	1.0000
Scots pine	AW − WD	1.0000	0.0012	0.3903	1.0000
Scots pine	AW − WW	1.0000	1.0000	0.0305	1.0000
Scots pine	AW − WR	1.0000	0.3759	0.0377	0.0002
Scots pine	WD − WW	1.0000	0.1135	0.5057	1.0000
Scots pine	WD − WR	1.0000	0.1033	0.5057	0.0005
Scots pine	WW − WR	1.0000	1.0000	0.8047	4.27×10^{-6}
Norway spruce	AD − AW	1.0000	1.0000	0.2359	0.0318
Norway spruce	AD − WD	0.2104	0.9113	1.0000	0.0568
Norway spruce	AD − WW	0.1965	1.0000	3.29×10^{-6}	0.0091
Norway spruce	AD − WR	0.0296	0.2210	0.0602	0.1080
Norway spruce	AW − WD	0.0404	1.0000	0.2228	8.18×10^{-7}
Norway spruce	AW − WW	0.0398	1.0000	0.2228	0.3750
Norway spruce	AW − WR	2.12×10^{-6}	1.0000	1.0000	0.3300
Norway spruce	WD − WW	1.0000	1.0000	4.15×10^{-8}	7.53×10^{-7}
Norway spruce	WD − WR	1.0000	1.0000	0.0193	7.69×10^{-7}
Norway spruce	WW − WR	1.0000	1.0000	0.2228	0.1080

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