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RESEARCH ARTICLE

Above- and below-ground phenology of four tree species in mixed forests and monospecific stands

Qiwen Guo[1] | Boris Rewald[2] | Hans Sandén[1] | Matthias Steinparzer[3] | Ramona Werner[1,4] | Douglas L. Godbold[1,2]

[1]Department of Ecosystem Management, Climate and Biodiversity, Institute of Forest Ecology, BOKU University, Vienna, Austria

[2]Department of Forest Protection and Wildlife Management, Faculty of Forestry and Wood Technology, Mendel University, Brno, Czech Republic

[3]Ecosystem Research and Environmental Information Management, Environment Agency Austria, Vienna, Austria

[4]School of Integrative Plant Science, Cornell University, Ithaca, USA

Correspondence
Douglas L. Godbold
Email: douglas.godbold@mendelu.cz

Qiwen Guo
Email: guoqiwennet@gmail.com

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Abstract

1. The phenology of tree root growth is poorly understood especially for mixed forests. To attempt to fill this knowledge gap, we selected four co-occurring broadleaf trees species of the European forests (*Quercus robur*, *Carpinus betulus*, *Tilia cordata* and *Acer platanoides*).
2. For these species growing in monocultures and mixtures, we monitored root growth, crown development and soil temperature over 2 years.
3. Our investigation revealed three key findings: (1) Root phenology showed species-specific patterns in monocultures and mixtures, with mixed stands showing patterns which reflected the dominate species. *Acer* and *Carpinus* showed earlier growth peak than species *Quercus* and *Tilia*. *Carpinus* and *Quercus* maintained winter root growth at temperatures as low as 4.2°C, whereas *Acer* initiated growth in spring at higher temperatures (8.7–8.9°C). (2) Above-ground and below-ground phenological events showed clear asynchrony which was significantly amplified in mixed stands compared to monocultures, with the interval between full leaf expansion and peak root production being longer in mixtures (approximately 5 weeks) than in monocultures (2–3 weeks). Root growth initiation consistently preceded leaf emergence in both monocultures and mixtures, but peak production occurred after full crown development. (3) Species mixing seasonally modified the temperature–growth relationship, with positive correlations observed in winter and negative correlations in summer.
4. Our findings demonstrate that the patterns of root growth modified in mixtures which cannot be predicted from the monocultures. These results highlight the importance of considering below-ground dynamics and species interactions when predicting forest ecosystem responses to climate change.

KEYWORDS
crown development, root dynamics, root growth, root phenology, tree diversity

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1 | INTRODUCTION

Plant phenology, the timing of key life cycle events, plays a critical role in ecosystem functioning and plant productivity. However, most research targets above-ground phenology, leaving roots comparatively understudied (McCormack, Dickie, et al., 2015; Nakahata, 2022; Radville et al., 2016; Steinaker et al., 2010; Withington et al., 2021). The lack of focus on roots is striking as roots comprise approximately 30%–50% of total plant production and regulate nutrient cycling and plant competition (Abramoff & Finzi, 2015; Moore et al., 2020; Rewald & Leuschner, 2009). Increasingly, studies have also shown a widespread above-below-ground phenological asynchrony (Abramoff & Finzi, 2015; Blume-Werry et al., 2016; Makoto et al., 2020; Nakahata, 2022; Steinaker et al., 2010). In temperate forests, for instance, root growth often precedes leaf emergence, potentially enabling plants to secure below-ground resources before competition for light intensifies during canopy closure (Guo, Rewald, et al., 2025; McCormack, Gaines, et al., 2015). However, how species interactions modify this root–shoot coordination remains poorly understood (Kharouba et al., 2018; Versace et al., 2021).

Mixed forests cover approximately 60% of the global forested area (Bravo-Oviedo et al., 2014; Pretzsch et al., 2017), yet most studies of root phenology focus exclusively on monospecific stands (Gaul et al., 2008; Nakahata, 2022; Quan et al., 2010). This represents a critical research gap, as mixtures can fundamentally alter both above- and below-ground processes (Guo, Kneeshaw, et al., 2025; Wardle et al., 2004; Werner et al., 2024). By changing canopy structure and morphology, mixtures create distinct microclimates (Atkins et al., 2023; Maurer et al., 2013; Zellweger et al., 2019) that change sub-canopy temperatures (De Frenne et al., 2019; Gillerot et al., 2024; Steinparzer et al., 2025; Zellweger et al., 2019) and, in turn, soil temperatures (Scherer-Lorenzen et al., 2005). Soil temperature is a key driver of fine root activity (Ding et al., 2020; Nakahata, 2022).

Species interactions in mixed forests are mediated through competitive and facilitative mechanisms, both of which can be expressed as changes in phenological coordination (Casper & Jackson, 1997; Leuschner et al., 2001; Rewald & Leuschner, 2009). Interspecific competition may promote temporal niche differentiation, with species adjusting their phenological timing to reduce overlap in resource competition (Alexander & Levine, 2019; Kalliokoski et al., 2010; McCormack, Gaines, et al., 2015). Conversely, facilitation can modify environmental responses through positive feedbacks such as microclimate amelioration or mycorrhizal networks (Callaway, 1995; Dickie et al., 2013; Kharouba et al., 2018; Van Der Heijden et al., 2008).

Despite the prevalence and importance of mixed forests, major knowledge gaps persist in how mixtures affect below-ground phenology (Radville et al., 2016; Withington et al., 2021). Evidence on the timing and coordination of root and shoot development is scarce because most phenology studies focus on single-species stands or above-ground traits (McCormack et al., 2014; Nakahata, 2022). Although above-ground phenology in mixtures may exhibit patterns that cannot be predicted from the component species (Versace et al., 2021), whether this extends below-ground is unknown. Critically, no comprehensive comparison of root phenology between monospecific and mixed stands exists (Gaul et al., 2008; Quan et al., 2010), limiting our ability to predict mixed forest responses to environmental change and to resolve the temporal mechanisms underlying their productivity benefits (Werner et al., 2024).

Given that species interactions can modify temporal resource availability and microclimate, we hypothesized the following: (1) root phenology is species-specific in monocultures, and mixtures induce measurable shifts in timing; (2) root growth initiates before leaf emergence across all species, with the magnitude of asynchrony varying among species and stand types; and (3) soil temperature affects root growth patterns, with mixtures modifying temperature–phenology relationships. This study provides the first comprehensive comparison of fine root phenology across monospecific and mixed stands of four common temperate broadleaf tree species (*Quercus robur*, *Carpinus betulus*, *Tilia cordata* and *Acer platanoides*) in Central European forests. These species co-occur widely in southern central Europe (Čarni et al., 2009), making them well suited for studying fine root dynamics in both monocultures and mixtures (Novák et al., 2023; Rewald et al., 2014). We quantify temporal patterns of root growth and mortality alongside soil temperature and leaf phenology, providing insights into how species interactions modify phenological processes and improve predictions of forest ecosystem responses to environmental and management change.

2 | MATERIALS AND METHODS

2.1 | Study area

The research was conducted in eastern Austria (48°19′2.989″ N, 16°4′0.613″ E), at the B-Tree experimental forest, Tulln an der Donau (Werner et al., 2024). The site has a mean annual temperature of 10.5°C and precipitation of 657 mm, with a moist Chernozem soil (pH 8.28, C:N ratio 19.7). The ~1.2 ha site was established in 2013 using 2-year-old seedlings obtained from Murauer Forstpflanzen (Orth im Innkreis, Austria). The design comprised seven plot types across three diversity levels, using four deciduous broadleaf species common in Central Europe: Norway maple (*Acer platanoides* L., Ap), small-leaved lime (*Tilia cordata* Mill., Tc), hornbeam (*Carpinus betulus* L., Cb) and pedunculate oak (*Quercus robur* L., Qr). Diversity levels were (i) four monocultures (Ap, Tc, Cb, Qr), (ii) two variants of two-species mixtures (*Quercus*/*Carpinus*, QrCb and *Acer*/*Tilia*, ApTc) and (iii) one four-species mixture (All; Figure S1). These species span resource-use strategies from highly acquisitive (Ap) to moderately acquisitive (Tc, Cb) to highly conservative (Qr) (de Rigo et al., 2016; Leuschner & Ellenberg, 2017). Acquisitive species have rapid resource acquisition and exhibit fast responses to environmental changes, while conservative species emphasize resource conservation and show more gradual, delayed responses (Royer et al., 2008). The species differ in mycorrhizal associations: ectomycorrhizal (EM) hosts (Cb, Qr), an EM and AM host (Tc) and an AM only host (Ap) (Dudka et al., 2023). Seedlings were planted in off-set

rows to achieve a uniform, hexagonal spacing of 1 m between trees (i.e. 1 m within rows, 0.87 m between rows), giving each tree six equidistant neighbours. In mixed plots, the tree species are not mixed in a regular pattern. This creates varied neighbourhood conditions for each tree, where individual trees may have different numbers of same-species (conspecific) or different-species (heterospecific) neighbours. Two-species mixtures contained 50% of each species; the four-species mixture contained 25% of each. To maintain uniform age and development, seedlings that died during the first two growing seasons (to autumn 2014) were replaced with trees of matching age and size.

2.2 | Root phenology, production and mortality

Root growth was monitored using 96 mini-rhizotron (MR) tubes installed vertically to 30 cm depth in autumn-winter 2021. Acrylic tubes (length 40 cm; inner diameter 64 mm; outer diameter 70 mm) were wrapped above-ground with black tape and capped to exclude light and rain. The investigation used 21 plots (seven plot types, each replicated three times) arranged in three blocks. Per replicate, monocultures had three MR tubes, two-species mixtures six and the four-species mixture eight. MR tubes were installed at the centre of the triangle formed by three trees (triplets). In the mixture, triplets were selected to represent all species combinations (e.g. in QrCb: triplets Qr-Cb-Cb and Qr-Qr-Cb).

Starting in January 2023 (day of year, DOY 23), approximately 16 months after MR tube installation, images were captured with a CI-600 In-Situ Root Imager (CID Bio-Science, Camas, USA) at 15-day intervals. The imager was positioned flush with the soil surface, producing images from 5 to 27 cm soil depth. The imager rotates within the tube to give an image 19.6 cm wide. Each image was divided horizontally, resulting in images of two soil depths, 5–16 cm and 16–27 cm. These two images were then further divided vertically to give a total number of four 11.3 by 9.8 cm images per MR tube, two per depth. For ease of reading, all decimal measurements were rounded to the nearest whole number throughout the manuscript (e.g. 16.3 cm to 16 cm). Over the entire experimental period, we analysed a total of 6912 images (18 months × 2 sampling times per month × 96 tubes × 2 depths). After 18 months, root imaging was suspended due to a malfunction of the imager.

Images were analysed in WinRHIZO Tron 2013 (Regent Instruments, Canada), where roots were manually traced (Richner et al., 2000). Fine root (diameter ≤2 mm) length was measured on each image. Individual roots were tracked through time, and cumulative root length density was calculated monthly (mm cm^{-2}). Newly appearing roots indexed production (mm cm^{-2} day^{-1}); roots that turned black or failed to reappear in two consecutive images indexed mortality (mm cm^{-2} day^{-1}) (Andersen et al., 2008; Bai et al., 2010; McCormack, Gaines, et al., 2015; Yuan et al., 2024). Root-growth peaks were defined as periods ≥30 days with production rates above the annual mean; DOY for peak growth was the mean across the three replicate plots per treatment.

To complete the second annual cycle for descriptive summaries, we applied the Holt-Winters additive model, which accounts for both trend and seasonal components in time series data (Chatfield & Yar, 1988). The model was fitted to the complete first year (12-month) series and used to forecast July–December of the second year based on the observed data from January to June 2024. Forecasted values were only utilized in calculating the relative percentage of the total annual root production (root production, %) and were excluded from all other statistical analyses.

Root species identification in mixtures used July 2023 MR images and species-specific morphological traits calibrated for this site (Werner et al., 2024). In that study, 160 soil cores were collected across all experimental plots, and fine roots were assigned to species using morphological criteria in Rewald et al. (2012). These established morphological profiles served as reference standards for our visual identification of roots in the MR images. Two trained observers independently classified roots; ambiguous cases (~7% of total root length) were excluded from species-specific analyses.

We assessed potential over- or underyielding effects by comparing observed root production and mortality in mixtures with expected values from monocultures. The expected values in mixtures were calculated as the species sum of yearly root production or mortality of the monoculture multiplied by the proportional abundance of each respective species in the mixture (Pretzsch & Schütze, 2009). For analyses of season, we defined spring as March–May, summer as June–August, autumn as September–November and winter as December–February.

2.3 | Leaf phenology

Leaf phenology (emergence, expansion, senescence, abscission) was tracked in 2023 and 2024 in parallel to the MR measurements. Leaf emergence was defined as the DOY at which new leaves break through the bud and become visible (change ≥50%), and was determined by observation. For canopy expansion, hemispherical photos were taken every 3–7 days on dry, windless days using an iPhone 13 in ultra-wide (fisheye) mode. The camera was positioned ~10 cm above the ground and aimed at the canopy above each MR tube. The camera was positioned in areas free of ground vegetation. Marker flags ensured identical framing and azimuth across dates. The images were processed using CAN-EYE v. 6.495 (INRAE, France) to quantify the number of green pixels (Demarez et al., 2008). The growth and changes in branches were considered negligible. We also engaged three independent observers to visually estimate and calibrate the image-based leaf expansion measurements. Full expansion was the first pair of consecutive measurements (mean DOY) with <5% change in leaf cover. Senescence onset was the first visible leaf coloration; abscission onset was the first leaves in litter traps (Steinparzer et al., 2022). All phenology dates were recorded as day of year (DOY).

2.4 | Soil temperature and canopy temperature

Soil temperature was measured using Thermochron iButtons (Maxim Integrated, San Jose, California, USA) installed at 5 cm and 15 cm

depths in November 2022. Sensors were calibrated at a common temperature and installed at the plot centres. Equipment malfunction and animal damage caused partial data loss in some plots (Figures S2 and S3). Missing data were treated as NA and handled appropriately within the mixed-effects models to maintain the balanced experimental design. Additional validation measurements confirmed that MR tubes did not significantly affect the soil temperatures adjacent to the tubes (Figure S4). Canopy temperature was measured at 1.5 and 3 m height at the centre of each plot using Thermochron iButton sensors mounted on wood poles. Sensors were shielded from direct solar radiation by white-painted covers to minimize heat absorption (Maclean et al., 2021). Temperature was logged every 2 h (soil) or 3 h (canopy) in two replicate plots per stand type.

2.5 | Statistical analysis

Prior to statistical analysis, data normality and homogeneity of variances were assessed using Shapiro–Wilk tests and Levene's tests, respectively.

2.5.1 | Root production and mortality analysis

Linear mixed-effects models (LMMs) were used to investigate the effects of tree species on root production and mortality at two soil depths (5–16 cm and 16–27 cm). Tree species were treated as a fixed effect, while experimental blocks were included as random effects to account for spatial variation within the experimental design. Model parameters were estimated using restricted maximum likelihood (REML). The significance of fixed effects was evaluated using F-tests with appropriate degrees of freedom. Model assumptions were verified through examination of standardized residuals for normality and homoscedasticity. When significant main effects were detected ($p < 0.05$), pairwise comparisons between tree species were conducted using estimated marginal means with Tukey's HSD adjustment, followed by Benjamini–Hochberg false discovery rate (FDR) correction to control the expected proportion of false discoveries among rejected hypotheses.

2.5.2 | Mixing effects analysis

To assess mixing effects while accounting for the variability inherent in both monoculture and mixture performance, we employed a net effect approach that avoids the statistical pitfall of treating monoculture means as fixed constants. To quantify the effects of species mixing on root production, we calculated net effects for each mixture by comparing observed production to expected values based on monoculture performance. For each tree species, we first established monoculture reference values by calculating the mean production from all monoculture replicates across the study period (e.g. mean Ap, mean Tc monoculture performance). We then computed the net effect for each individual mixture plot as the difference between observed mixture production and expected production, where expected production was calculated as the sum of each species' monoculture means multiplied by its proportional representation in the mixture. To test whether mixing effects were significantly different from zero, we performed one-sample *t*-tests on the distribution of net effects. *p*-values were adjusted using Holm–Bonferroni correction to control for family-wise error rate across multiple mixture types and years.

2.5.3 | Temperature–root–growth relationships

The relationship between root growth dynamics and soil temperature was quantified using Pearson's correlation coefficients. Differences in soil temperature between mixed and monoculture stands were analysed using one-way ANOVA. When significant main effects were detected, pairwise comparisons were conducted using Tukey's HSD test, with additional FDR correction using the Benjamini–Hochberg procedure. All analyses were performed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA), with the significance level set at $p < 0.05$. Figures were drawn using Python (version 3.8).

3 | RESULTS

Our investigation revealed three key findings related to our hypotheses: (1) root phenology showed species-specific patterns in monocultures and mixtures, with mixed stands showing patterns that reflected the dominant species; (2) above- and below-ground phenological events were asynchronous, with asynchrony significantly greater in mixtures—the interval between full leaf expansion and peak root production being longer in mixtures (~5 weeks) than in monocultures (2–3 weeks); and (3) species mixing seasonally modified the temperature–root–growth relationship, with positive correlations observed in winter and negative correlations in summer.

3.1 | Root phenology in monocultures versus mixtures

3.1.1 | Species composition of root systems in mixed stands

Species-level root identification revealed strongly asymmetric contributions to total root length in mixtures, with larger soil depth-dependent shifts in the four-species mixture (Figure 1).

In the upper soil layer (5–16 cm), *Acer platanoides* constituted 80% of the total root length in ApTc mixtures and dominated the four-species mixture to the same extent. In the deeper soil layer (16–27 cm), *Acer* maintained its dominance in ApTc mixtures but showed slightly reduced presence in the four-species mixture. In contrast, *Quercus robur* represented only 195–28% of the roots in QrCb mixtures, with a modest increase at the deeper layer.

3.1.2 | Root production patterns

As production represents root length accumulated since the prior observation, the first growth appears at DOY 53 (initial observation at DOY 23; Figure 2). Root growth had distinct temporal patterns that varied by species, soil depths and stand types.

At 5–16 cm soil depth, monocultures exhibited species-specific root production patterns with clearly defined seasonal peaks (significant increases above the annual mean value). Ap and Cb showed an initial peak early in 2023 (DOY 53), Qr and Tc peaked in spring (DOY 83), and summer peaks occurred for Ap and Cb on DOY 173 and for Qr and Tc later on DOY 203. Root production patterns observed in mixtures primarily reflected the dominant species, with timing and magnitude modified by interspecific interactions (Figure 2). In the ApTc mixture, where *Acer* contributed ~80% of roots, peaks occurred at DOY 53, 173, and 233, mirroring Ap. In QrCb, dominated by *Carpinus* (725–81% of roots), main production peaks were at DOY 83 and 173; notably the Cb-specific peak seen on DOY 113 in monoculture was absent. The four-species mixture, with 81% *Acer* representation in the upper layer, showed production patterns mostly similar to the Ap monoculture. At 16–27 cm depth, similar dominance patterns emerged, but with fewer growth peaks (Figure 2). The root production patterns in mixtures largely paralleled those at shallower depths, though with slightly increased representation of *Quercus* in QrCb and decreased dominance of *Acer* in the four-species mixture (see Figure 1).

Winter root growth (December 2023 to February 2024) revealed important species-specific differences and mixture effects (Figure 2). In monocultures, Ap and Tc ceased root growth during winter (DOY 353), while Qr and Cb monocultures maintained minimal but continuous root production when soil temperatures were approximately 4.2–4.5°C (Figures S2 and S3). However, all mixtures maintained continuous winter root production, suggesting a facilitative effect of mixing on winter root activity.

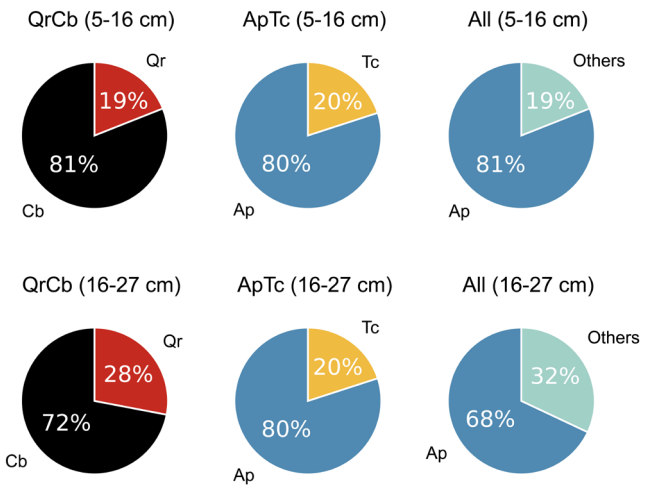

FIGURE 1 Species-specific root length proportion (%) in two-species (QrCb, ApTc) and four-species (All) tree mixtures, based on mini-rhizotron observations at two depths (5–16 cm; 16–27 cm). *Quercus robur* (Qr), *Carpinus betulus* (Cb), *Acer platanoides* (Ap) and *Tilia cordata* (Tc); 'Others' refers to Cb, Qr, and Tc in 'All'.

3.1.3 | Root mortality patterns

Root mortality exhibited species-specific timing in monocultures (Figure 3). At 5–16 cm soil depth in 2023, each species showed characteristic mortality peaks (e.g. Cb at DOY 53, 113 and 323; Qr at DOY 83 and 323). In mixtures, mortality patterns diverged from expectations based on component species (Figure S5). For instance, the ApTc mixture showed only one of the three expected mortality

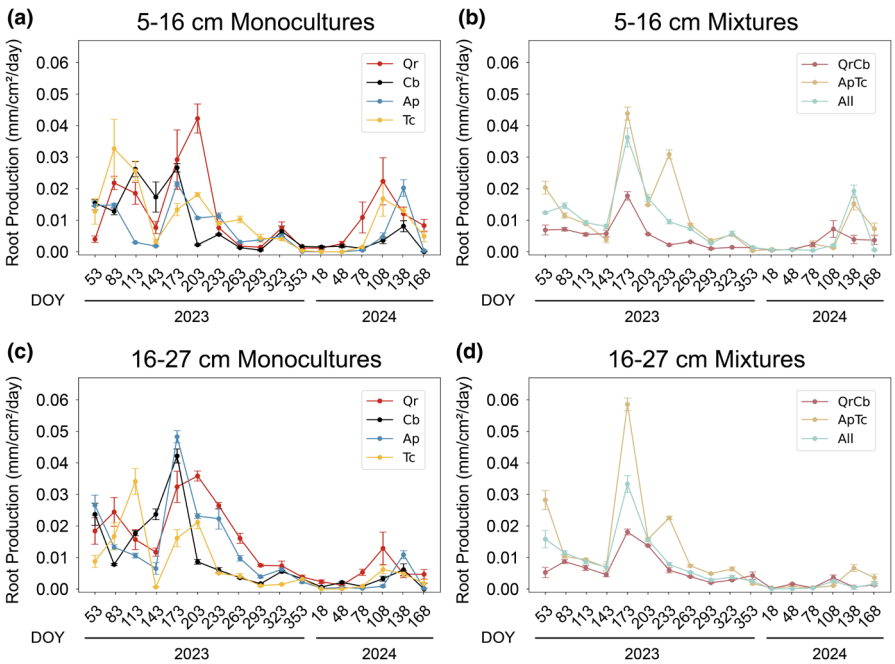

FIGURE 2 Root production at two soil depths for four tree species growing in (a, c) monocultures and (b, d) mixtures. Species/mixtures: *Quercus robur* (Qr), *Carpinus betulus* (Cb), *Acer platanoides* (Ap) and *Tilia cordata* (Tc) monocultures, two-species (QrCb, ApTc) and four-species (All) mixtures. Shown are means of newly produced root lengths from three replicate plots of each species or mixtures. Error bars indicate SE. The *x*-axis shows the day of year (DOY). The first data point appears at DOY 53 because values represent net growth since the preceding observation (initial observation in the year 2023: DOY 23).

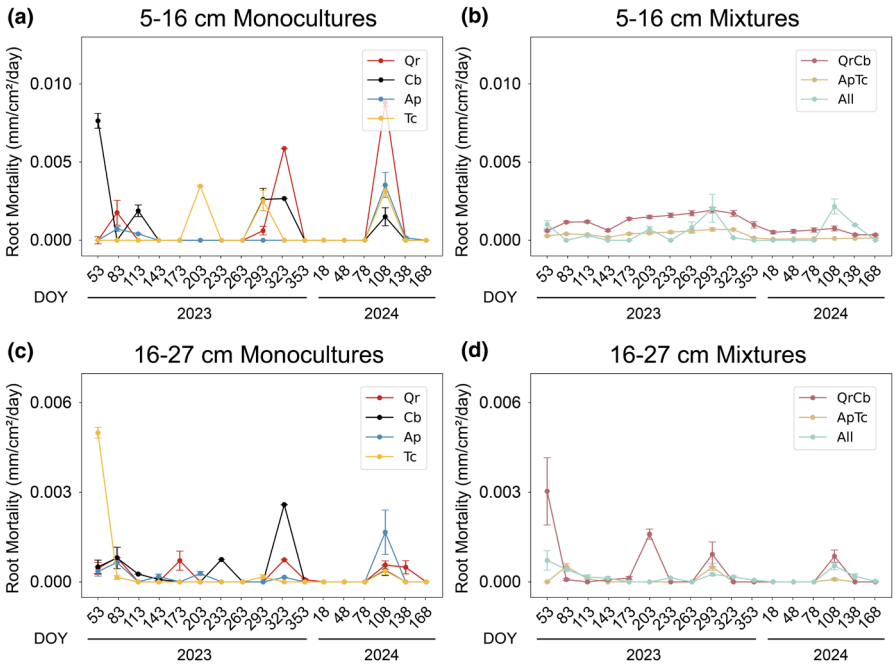

FIGURE 3 Root mortality at two soil depths for four tree species growing in (a, c) monocultures and (b, d) mixtures. Species/mixtures: *Quercus robur* (Qr), *Carpinus betulus* (Cb), *Acer platanoides* (Ap) and *Tilia cordata* (Tc) monocultures, two-species (QrCb, ApTc) and four-species (All) mixtures. Shown are the mean root mortality from three replicate plots of each species or mixtures. Error bars indicate SE. The *x*-axis shows the day of year (DOY). The first data point appears at DOY 53 as values represent net growth since the preceding observation (initial observation in the year 2023: DOY 23). There are differences in y-axis scale between soil depths.

peaks, while QrCb mixtures displayed three peaks despite only two being predicted from monoculture patterns (Figure S5).

3.2 | Annual root production and mortality in mixtures versus monocultures

Annual fine root production showed several significant differences between species and stand types, with Qr monoculture significantly higher than other species; however, QrCb presented the lowest root production (Figure 4a,c). Annual fine root mortality was significantly higher at 5–16 cm compared to 16–27 cm soil depth in most treatments, with total mortality in the upper layer approximately twice that of the lower layer (Figure 4b,d). Ap monoculture showed the lowest root mortality among all plots, while other stand types showed no significant differences.

Comparing observed with abundance-weighted expectations from monocultures, annual root production exhibited significant underyielding in QrCb mixtures at both soil depths, whereas ApTc and the four-species mixture did not differ from expectation (Figure 5a,c). Annual mortality in QrCb and ApTc was slightly higher than expected but not significant at either soil depth (Figure 5b,d).

3.3 | Above- and below-ground phenological asynchrony

In 2023, leaf emergence began with Cb and Ap (DOY 82), followed by Qr and Tc (DOY 86) (Table S1). Full leaf expansion varied considerably among species, occurring earliest in Cb (DOY 130) and latest in Tc (DOY 168). In 2024, emergence patterns were similar, but generally earlier than in 2023, with full expansion achieved approximately 11 days earlier across species (Table S1). Importantly, growing in mixture did not significantly alter the timing of leaf emergence or full expansion for any species, indicating that above-ground phenology was primarily driven by species-specific responses to environmental cues rather than by interspecific interactions.

Root growth initiation consistently preceded leaf emergence in spring for all species in both monocultures and mixtures (Figure 6). However, peak root production typically occurred after full leaf expansion, ~3 weeks later in monocultures (except Qr) and ~5 weeks later in mixtures during 2023 (Figure 6). In 2024, the pattern persisted, with peak root production occurring about 2 weeks after full leaf expansion in monocultures and 5 weeks after in mixtures. Root mortality showed distinct seasonal peaks in monocultures during late summer (DOY 220-280), coinciding with leaf senescence and abscission periods, while mixtures maintained consistently low mortality rates with minimal seasonal variation (see Figure 3, Table S1). These results show persistent above–below-ground asynchrony, amplified in mixtures relative to monocultures.

3.4 | Phenology related to air and soil temperature

3.4.1 | Temperature thresholds for phenological events

Leaf emergence occurred at daily mean canopy temperatures (3 m) of 7.8–7.9°C in 2023 and 9.7–9.9°C in 2024, with full expansion achieved at 14.1–14.2°C and 16.3–16.4°C, respectively (Figure 6, Figures S5 and S6).

Peak root production occurred at 13.0–13.2°C for Ap, Tc and Cb, except for Qr where peak production occurred at 16.7–16.8°C

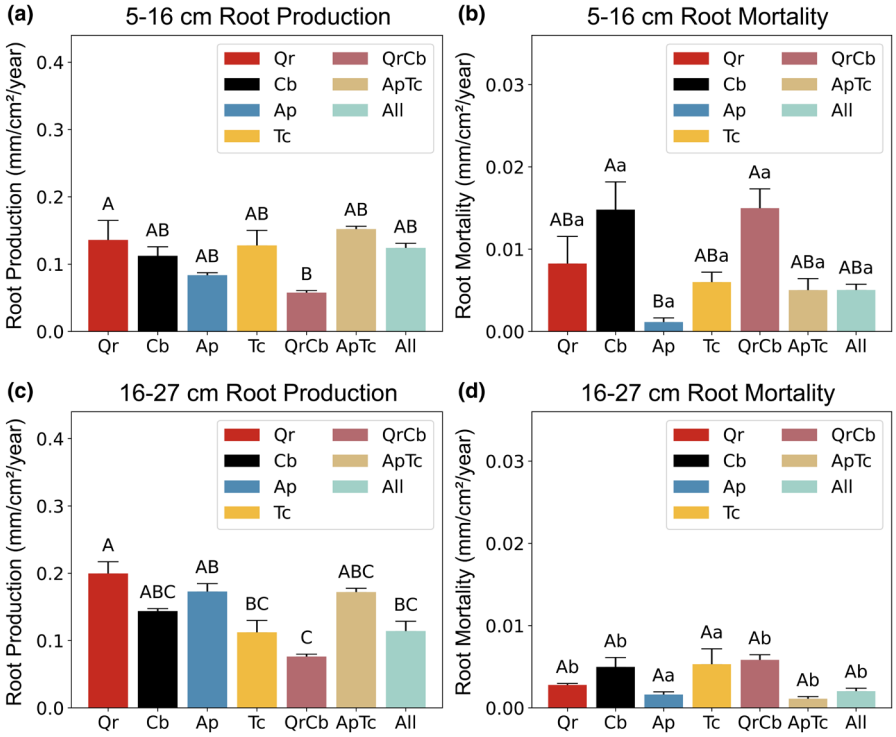

FIGURE 4 Annual (a, c) root production and (b, d) root mortality at two soil depths for four tree species growing in monocultures and mixtures. Species/mixtures: *Quercus robur* (Qr), *Carpinus betulus* (Cb), *Acer platanoides* (Ap) and *Tilia cordata* (Tc) monocultures, two-species (QrCb, ApTc) and four-species (All) mixtures. Shown are the means from three replicate plots of each species or mixtures. Error bars show the SE. Uppercase letters indicate significant differences between plot types; lowercase letters indicate significant differences among depths. There are differences in *y*-axis scale between production and mortality.

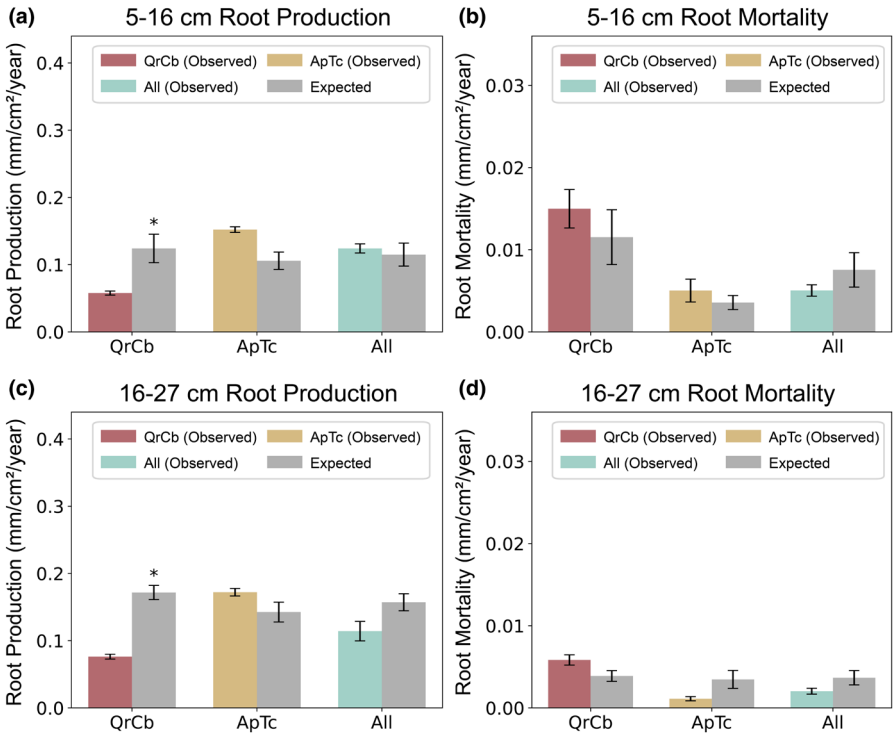

FIGURE 5 Observed (coloured) and expected (grey) annual sums of (a, c) root production and (b, d) root mortality in two-species (*Quercus/Carpinus*, QrCb; *Acer/Tilia*, ApTc) and four-species (All) tree mixtures. Expected values are abundance-weighted sums of monoculture means of the composing species (see Section 2). Asterisks indicate significant differences between observed and expected values.

(Figure 6, Figures S2 and S3). In 2024, Ap and Tc root growth initiated at higher temperatures in February (8.7–8.9°C), while Qr and Cb maintained growth throughout winter (December 2023 to February 2024) at temperatures as low as 4.2–4.5°C.

These distinct temperature thresholds for root versus leaf phenology further highlight the asynchrony in above-ground and below-ground development.

3.4.2 | Seasonal correlation between root growth and soil temperature

Root production showed distinctive seasonal correlations with soil temperature that varied by species, mixture types and soil depths (Figure 7). During winter, Qr and Cb monocultures exhibited strong

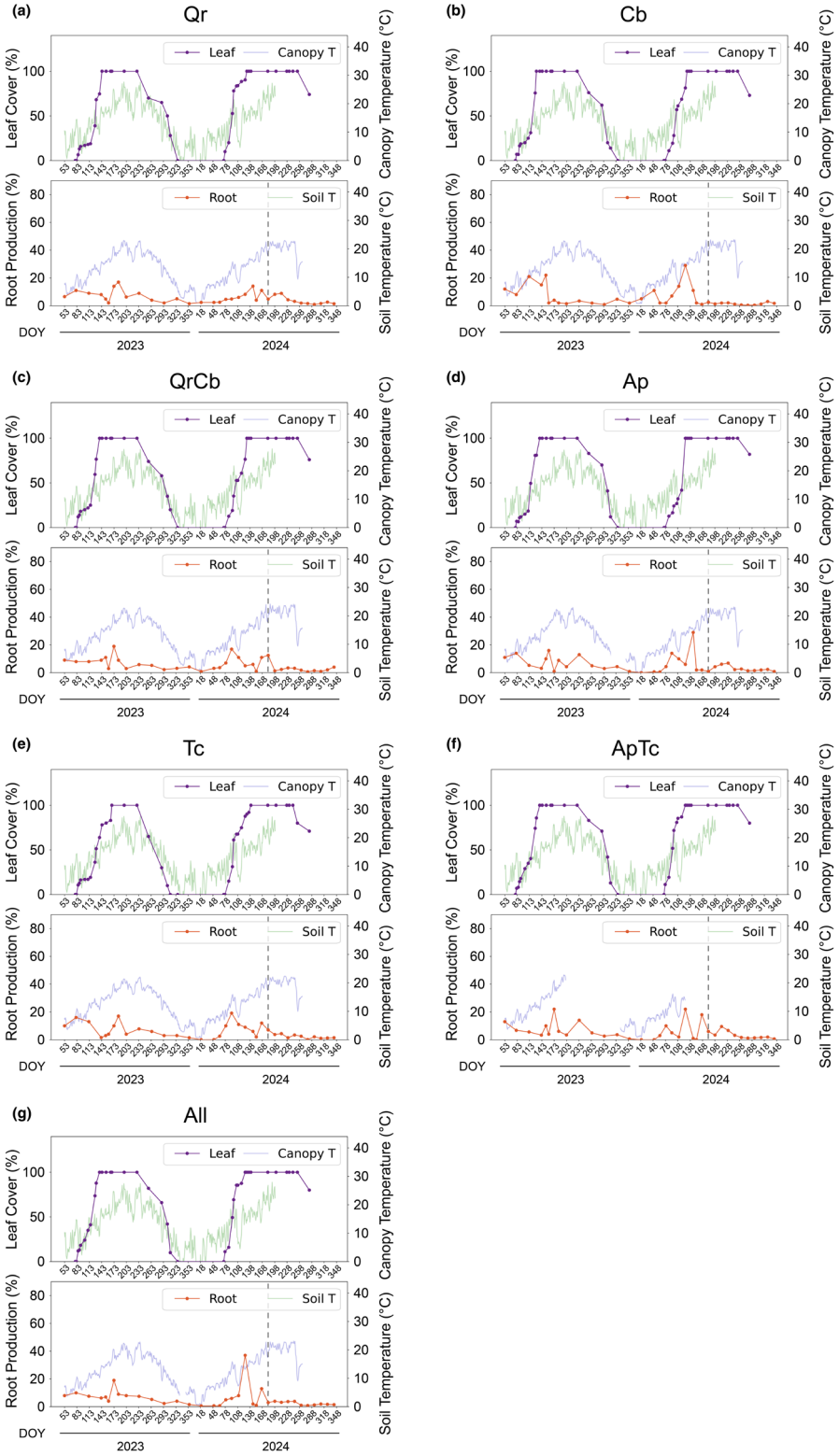

FIGURE 6 Seasonal root production (5–27 cm soil depth) and leaf cover of *Acer platanoides* (Ap), *Tilia cordata* (Tc), *Quercus robur* (Qr) and *Carpinus betulus* (Cb) growing in monocultures (a–d), two-species (*Acer/Tilia* Ap/Tc or *Quercus/Carpinus* Qr/Cb) (e, f) and four-species (All) mixtures (g). The purple line indicates percentage of maximum leaf cover; when consecutive observations showed changes <5%, leaf expansion was considered complete and the percentage was set to 100%. The red line indicates root growth, with values representing the percentage of total annual production. Data after the dashed line are simulated (starting from July 2024). The daily soil temperature (15 cm depth) and daily canopy temperature (3 m height) are indicated by mauve and green lines, respectively.

positive correlations between root production and soil temperature at both 5 and 15 cm depths, maintaining growth activity even at low temperatures. In spring, when root growth resumed in Ap and Tc monocultures, these species also showed strong positive correlations with temperature.

Interestingly, all mixtures demonstrated positive correlations with soil temperature at 5 cm depth during winter, including mixtures containing Ap and Tc (four-species mixtures), which showed no growth in Ap and Tc monoculture during this period. This suggests that higher mixing levels may facilitate winter root activity in Ap and Tc. At 15 cm depth, however, this positive correlation was absent in QrCb and four-species mixtures, indicating depth-specific responses to mixing.

Seasonal shifts in temperature correlations were evident across treatments. During spring, only the four-species mixture maintained

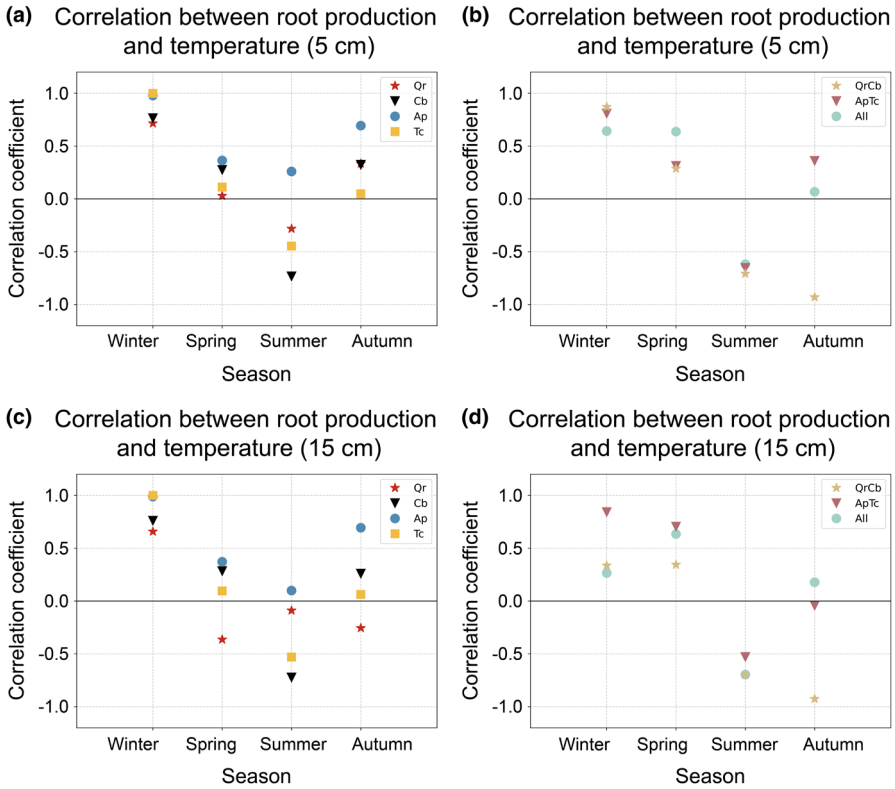

FIGURE 7 Seasonal correlations (Pearson's product–moment) between fine root production (5–16 cm) and soil temperature for four tree species growing in (a, c) monocultures and (b, d) mixtures. Species/mixtures: *Quercus robur* (Qr), *Carpinus betulus* (Cb), *Acer platanoides* (Ap) and *Tilia cordata* (Tc) monocultures, two-species (QrCb, ApTc) and four-species (All) mixtures. Correlations $|r| \geq 0.5$ are considered significant, with stronger relationships as values approach ±1. Seasons: Winter (December–February), spring (March–May), summer (June–August) and autumn (September–November).

strong positive correlations with soil temperature at both depths, while ApTc mixtures showed strong positive correlations specifically at 15 cm soil depth. Summer revealed a marked shift, with Cb monoculture and all mixtures demonstrating strong negative correlations at 5 cm depth, suggesting potential heat stress or moisture limitations at higher temperatures. Similar negative correlations were observed at 15 cm depth for Tc and Cb monocultures and all mixtures. In autumn, contrasting patterns emerged, with Ap monoculture showing positive correlations at both depths, while QrCb mixtures exhibited strong negative correlations. These shifting correlations throughout the growing season highlight the complex and dynamic relationship between root growth and soil temperature, and how species mixing may substantially modify these temperature–growth relationships.

4 | DISCUSSION

4.1 | Patterns of root production and mortality in mixed versus monospecific stands

Species mixing caused altered temporal patterns of both root production and mortality. The changes in temporal patterns resulted in considerable annual cumulative effects between different species combinations. QrCb mixtures exhibited significant underyielding in root production at both soil depths while maintaining expected mortality rates (Figure 5). In contrast, ApTc mixtures showed no significant differences from expected values in either production or mortality (Figure 5). These findings align with the variable results reported in previous studies, where underyielding (Szwagrzyk & Gazda, 2007; Vilà et al., 2003), neutral effects (Bauhus et al., 2000; Leuschner et al., 2001), and overyielding (Ma & Chen, 2016; Zhang et al., 2012) of fine root biomass have all been observed in mixed stands. The mixture-specific responses suggest that species identity and combination, not merely diversity, are critical factors in determining below-ground responses to mixing.

The species composition proportions in mixed stands derived from our visual identification were consistent with the biomass distribution patterns previously quantified in soil cores from the same experimental plots (Werner et al., 2024). The asymmetric contribution explains the significant underyielding observed in QrCb mixtures, as *Quercus*, the more productive species in monoculture, was substantially suppressed when grown with *Carpinus*. The finding that acquisitive species (particularly *Acer*) dominated the root zone in mixtures, regardless of showing lower production in monocultures than other species, challenges assumptions about below-ground competitive hierarchies in temperate forests. Interestingly, *Quercus robur* had higher fine root production among all species (Figure 4), yet this did not translate into an overall competitive advantage in mixtures (Werner et al., 2024). This observation highlights an important aspect of our phenological investigation: despite *Quercus*'s high investment in below-ground resources (Barbaroux et al., 2003; Ramírez-Valiente et al., 2018), the timing of *Quercus* root growth relative to other species may be more critical for competitive interactions than absolute production values (Casper & Jackson, 1997; Kalliokoski et al., 2010; Rewald & Leuschner, 2009). The continued winter root activity we observed in *Quercus*, combined with its relatively late leaf expansion, suggests a temporal resource strategy that prioritizes securing below-ground resources during periods when

competition from acquisitive species is reduced. In mixed stands, however, the presence of other species with different phenological patterns appears to modify this temporal advantage, potentially explaining why high root production alone did not confer competitive dominance (Casper & Jackson, 1997; Kressuk et al., 2025; Leuschner et al., 2001; Rewald & Leuschner, 2009).

4.2 | Root–shoot phenological asynchrony in monocultures and mixed stands

Our investigation confirmed consistent asynchrony between above- and below-ground phenology across all species, with root growth preceding leaf emergence in both monocultures and mixtures. This pattern agrees with observations from previous studies in temperate forests (Ding et al., 2020; Makoto et al., 2020; McCormack, Gaines, et al., 2015), but contrasts with findings where root growth followed leaf emergence (Burke & Raynal, 1994; Du & Fang, 2014; Schwieger et al., 2019). The observed spring root growth appeared decoupled from leaf photosynthesis (Ding et al., 2020; Makoto et al., 2020; McCormack, Gaines, et al., 2015; Nakahata, 2022), suggesting that initial root growth might have relied on stored non-structural carbohydrates (Barbaroux et al., 2003; Joslin et al., 2001; Martínez-Vilalta et al., 2016; Tierney et al., 2003). Interestingly, different species exhibited distinct patterns of phenological coordination. *Acer platanoides* and *Carpinus betulus*, characterized by early leaf emergence and expansion, showed a depression in root growth during their leafing period (Figure 6). This pattern suggests possible carbon allocation trade-offs between root and leaf development (Lambers & Oliveira, 2019; Poorter et al., 2012; Walter & Schurr, 2005). Such depression was notably absent in *Quercus robur* and *Tilia cordata*, which displayed significantly later leaf development. These differences potentially reflect divergent resource allocation strategies between acquisitive (*Acer*) and conservative (*Quercus*) functional types.

While leaf phenology remained remarkably consistent across monocultures and mixtures, root phenology showed pronounced plasticity in mixed stands. Notably, root growth patterns in mixed stands primarily reflected the dominant species (Figures 1 and 2), with mixtures displaying different growth peaks compared to what would be expected from the component species growing in monoculture. Additionally, the interval between peak root growth and complete leaf expansion differed between monocultures (approximately 3 weeks) and mixtures (approximately 5 weeks) (Table S1, Figure 6). These observations suggest that interspecific interactions may influence temporal resource allocation patterns below-ground more readily than above-ground.

4.3 | Correlations between environmental factors and root phenology

Our results showed that root growth was associated with specific soil temperature ranges, though these relationships varied seasonally and by species. During winter, all four species exhibited positive correlations between root growth and soil temperature (Figure 7). Notable species differences emerged in winter activity; *Quercus* and *Carpinus* maintained continuous root growth at temperatures as low as 4.2–4.5°C during winter, whereas *Acer* and *Tilia* showed complete cessation until temperatures reached 8.7–8.9°C in February (Figure 6, Figures S2 and S3). These thresholds are consistent with previous reports for other temperate species, where root growth continued at soil temperatures between 2 and 4°C (Alvarez-Uria & Körner, 2007; Marchand et al., 2025; Quan et al., 2010; Schenker et al., 2014) or even approaching 0°C (Malyshev et al., 2023; Marchand et al., 2025). These contrasting thermal strategies may reflect differences in mycorrhizal associations, with EM species (*Quercus* and *Carpinus*) maintaining winter activity while AM species (*Acer*) require warmer conditions. The temperature–growth relationship changed seasonally, with positive correlations becoming less pronounced in spring and shifting to negative correlations in summer (Figure 7), particularly in the upper soil layers. This seasonal shift may reflect temperature stress at higher summer temperatures, as suggested by previous studies (McCormack & Guo, 2014; Nakahata, 2022; Tryon & Chapin III, 1983). We observed an earlier onset of peak summer root growth in 2024 relative to 2023 in all species (Figure 2), coinciding with earlier soil warming. In 2024, soil temperatures reached approximately 15°C by day 110, while this temperature was only reached around day 142 in 2023 (Figures S2 and S3). Notably, 15°C was associated with peak root growth in both years, suggesting that specific temperature thresholds may be associated with intensified root growth regardless of calendar date.

Soil depth emerged as another significant factor associated with root growth and mortality patterns. More frequent root growth and mortality events were observed at 5–16 cm compared to 16–27 cm depth (Figures 2 and 3). This vertical stratification agrees with previous research indicating that roots in the upper 20 cm of soil are more responsive to environmental fluctuations (Germon et al., 2016; Luo et al., 2021; Maeght et al., 2015; Wang et al., 2022). A significant limitation of our study is the lack of soil moisture measurements, which represent a critical cofactor in root growth regulation that often interacts with temperature effects. The seasonal shifts in temperature-growth correlations we observed, particularly the negative correlations during summer months, may be substantially influenced by soil moisture dynamics rather than temperature alone. During summer periods, high temperatures typically coincide with reduced soil moisture, creating potential confounding effects in our temperature-based interpretations. For instance, the negative correlations between root growth and temperature observed in summer at 5 cm depth for *Carpinus* and all mixtures might primarily reflect drought stress responses rather than direct temperature inhibition effects (Brunner et al., 2015; Nikolova et al., 2020).

The interplay between soil temperature and moisture is particularly complex in temperate forest ecosystems, where fine root growth can be simultaneously limited by both factors with

varying temporal dominance (Hertel et al., 2013; Lopez et al., 1998; Nakahata, 2022; Pregitzer et al., 2000; Wang et al., 2021). In spring, when soil moisture is generally abundant, temperature likely acts as the primary growth-limiting factor, explaining the positive correlations we observed. Conversely, in summer, soil moisture typically becomes the limiting factor, potentially explaining the shift to negative temperature correlations as higher temperatures accelerate soil drying. The observed phenological synchrony or asynchrony coincided with differences in root production–soil temperature relationships between mixtures. These findings underscore the importance of considering not just the magnitude but also the timing of above- and below-ground resource allocation when evaluating species interactions in mixed forests. The complex interspecific interactions observed in root growth patterns, where mixtures primarily reflected dominant species dynamics despite species-specific patterns in monocultures, suggest that further investigation is needed with simultaneous monitoring of multiple environmental factors beyond temperature alone.

5 | CONCLUSION

Our research demonstrates that species mixing leads to highly asymmetric below-ground contribution, with acquisitive species (particularly *Acer*) dominating the root zone despite conservative species showing higher production in monocultures. This competitive asymmetry varied with soil depth, with conservative species showing slightly increased presence in deeper soil layers. Root growth patterns in mixtures clearly reflect the growth of the dominant species; however, changes in the patterns of both root production and mortality were found in mixtures compared to the monocultures independent of the dominant species. These findings highlight the importance of species-specific root identification across soil depths in mixed forest studies and reveal that below-ground competitive dynamics may differ substantially from expectations based on monoculture performance.

AUTHOR CONTRIBUTIONS

Douglas L. Godbold, Boris Rewald, Hans Sandén, Qiwen Guo, Matthias Steinparzer and Ramona Werner planned and designed the research. Qiwen Guo collected and analysed the data. Qiwen Guo and Douglas L. Godbold wrote the manuscript. Qiwen Guo, Douglas L. Godbold, Hans Sandén and Boris Rewald reviewed and edited the manuscript, with input from all co-authors. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Figshare Digital Repository: https://doi.org/10.6084/m9.figshare.30272512.v1 (Guo, 2025).

ORCID

Qiwen Guo https://orcid.org/0000-0002-5436-5932
Boris Rewald https://orcid.org/0000-0001-8098-0616
Hans Sandén https://orcid.org/0000-0002-2496-6307
Matthias Steinparzer https://orcid.org/0000-0002-0389-843X
Ramona Werner https://orcid.org/0000-0003-0075-1720
Douglas L. Godbold https://orcid.org/0000-0001-5607-5800

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Map of the species distribution in B-Tree Experimental Plots. The four species (*Acer platanoides L.* (Ap), *Tilia cordata Mill.* (Tc), *Quercus robur L.* (Qr), *Carpinus betulus L.* (Cb)) are replicated as monocultures, two species mixture ApTc and QrCb and as four species mixture All.

Table S1. Phenological events of leaf in *Acer platanoides* (Ap), *Tilia cordata* (Tc), *Quercus robur* (Qr), and *Carpinus betulus* (Cb) growing in monocultures or in two-species (*Acer/Tilia* Ap/Tc, or *Quercus/Carpinus* Qr/Cb) and four-species (All) mixtures.

Figure S2. Soil temperature in 5 cm depth of different species and diversity level. The maximum daily soil temperature is indicated by the red line, the minimum daily soil temperature is indicated by the green line, the average daily soil temperature is indicated by the blue line.

Figure S3. Soil temperature in 15 cm depth of different species and diversity level. The maximum daily soil temperature is indicated by the red line, the minimum daily soil temperature is indicated by the green line, the average daily soil temperature is indicated by the blue line.

Figure S4. Daily soil temperature in the immediate vicinity of minirhizotron (MR) tubes and ambient daily soil temperature. The red line indicates the ambient daily soil temperature, the green line indicates the daily soil temperature in the immediate vicinity of minirhizotron (MR) tubes.

Figure S5. Observed and expected curve of root production and root mortality in two-species (*Acer/Tilia* Ap/Tc, or *Quercus/Carpinus* Qr/Cb) and four-species (All) mixtures. The green dashed line represents

the expected curve for root production, and the yellow dashed line represents the expected curve for root mortality.

Figure S6. Canopy temperature in 1.5 m height of different species and diversity level. The maximum daily canopy temperature is indicated by the red line, the minimum daily canopy temperature is indicated by the green line, the average daily canopy temperature is indicated by the blue line.

Figure S7. Canopy temperature in 3 m height of different species and diversity level. The maximum daily canopy temperature is indicated by the red line, the minimum daily canopy temperature is indicated by the green line, the average daily canopy temperature is indicated by the blue line.

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