



Strategies for difficult times: physiological and morphological responses to drought stress in seedlings of Central European tree species

Marzena Niemczyk¹ · Barb R. Thomas² · Szymon Jastrzębowski¹

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Abstract

Key message *Picea abies* and *Pinus sylvestris* seedlings conserve soil water and are more sensitive to drought showing photoinhibition even under moderate stress, while *Quercus robur* and *Fagus sylvatica* seedlings have higher soil water uptake, they show photoinhibition only under severe drought.

Abstract Drought is an important factor in ecological change and species distribution shifts. We conducted a greenhouse experiment with seedlings of four Central European tree species: *Pinus sylvestris* (PS), *Picea abies* (PA), *Fagus sylvatica* (FS), and *Quercus robur* (QR) to investigate their response to drought. We monitored maximum quantum yield of photosystem II (F_v/F_m) during a 60-day drought treatment and measured above- and below-ground characteristics as morphophysiological responses to drought stress. Due to the fast, juvenile growth of the deciduous species (FS and QR), they had higher soil water uptake and suffered more quickly from severe drought than conifers (PS and PA). The deciduous species maintained a higher F_v/F_m , until volumetric water content (VWC) was very low (<5%), oscillating within a narrow safety margin. Both conifers PA and PS conserved soil water; photoinhibition in these species occurred at VWC of 14.5% and 5.5%, respectively. There were no differences in height between drought-stressed and irrigated seedlings, while drought reduced all root characteristics of the deciduous seedlings. Our study revealed trade-offs between different water management strategies, growth rate, and photoinhibition during the juvenile growth stage of our focal species. For climate change adaptation, anisohydric deciduous tree species seem to be more suitable. However, PS, with its water-conserving management and low photoinhibition threshold, holds promise for successful regeneration on drought-prone sites. Since species selection is critical for forest sustainability, our study contributes to the broader discussion of tree species' drought resistance during the vulnerable juvenile phase in the face of climate change.

Keywords Drought stress · Isohydric · Anisohydric · Maximum efficiency of PSII · Chlorophyll fluorescence · Root characteristics

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✉ Marzena Niemczyk
M.Niemczyk@ibles.waw.pl

¹ Department of Silviculture and Forest Tree Genetics, Forest Research Institute, Braci Leśnej 3, Sękocin Stary, 05-090 Raszyn, Poland

² Department of Renewable Resources, University of Alberta, 442 Earth Sciences Building, Edmonton, AB T6G 2E3, Canada

Introduction

The recent general circulation models of the World Climate Research Programme (CMIP6 models) predict that global warming will affect the hydrological cycle, and increase the risk and severity of droughts in some regions of the world, by the end of the century (Cook et al. 2020). Concomitantly, soil drying is predicted to become more widespread, with the severity increasing with rising temperatures (Cook et al. 2020). Sensitivity to drought, temperature, and other climatic conditions (e.g., length of the growing season) is critical to the geographic distribution of individual species and their communities (Engelbrecht et al. 2007; Dyderski et al. 2018). The distribution of plants will be significantly

impacted by these environmental changes, but the magnitude of these effects will vary, depending on the species' tolerance for water scarcity (Schipper et al. 2020; Sandoval-Martínez et al. 2023). Nearly half of the Earth's terrestrial surface is covered by forests (Melillo et al. 1993), and the dominant forest-forming tree species play a key role by providing habitat, food, or mutualisms with many animals, fungi, microorganisms, and other plants (Aitken et al. 2008). Therefore, a transition in the dominant tree species due to climate change may lead to changes in the characteristics of entire ecosystems and dependent organisms (Wulf and Naaf 2009; Lindner et al. 2010; Choat et al. 2012; Ellis 2013; Dyderski et al. 2018; Remke et al. 2022). Changes in forest communities will also affect the various services that forests provide for humans. Understanding the mechanisms of drought tolerance/avoidance and the effects of drought on trees is, therefore, critical for the appropriate management of forest resources, selection of the best species and provenances in planted forests, and conservation of forest ecosystems (Robakowski et al. 2020).

Given trees' long lifespans, adaptation to a water-scarce environment was one of the first challenges in their evolution, leading to the development of strategies and mechanisms to cope with water deficits (Aranda et al. 2012). These strategies include changes in root systems, hydraulic architecture, and/or stomata conductance regulation (Martínez-Sancho et al. 2017). Stomata are the primary regulators of water fluxes in the soil–plant–atmosphere continuum (Urban et al. 2017). Stomatal closure leads to an improvement in the water balance and water status of the plant by limiting transpiration losses. However, due to drought, the cost of stomata closure is a limitation of CO₂ uptake into chloroplasts, a reduction in photosynthesis and growth, and subsequently an increased risk of photooxidative stress (Urban et al. 2017). In extreme cases, prolonged drought can lead to tree death due to carbon starvation (long stomata closure) or hydraulic failure (McDowell et al. 2008).

Recent studies (Salmon et al. 2020) have laid the foundation for incorporating stomatal and non-stomatal limitations on photosynthesis in response to drought stress. Recently, a strong relationship between the chlorophyll fluorescence ratio and net photosynthesis under drought stress was also established, making chlorophyll fluorescence (ChlF) a powerful tool for monitoring the photosynthetic properties of terrestrial ecosystems under drought stress (Zhuang et al. 2020), although the kinetics of chlorophyll fluorescence induction has been routinely used for many years to provide information on photosynthetic performance (Lichtenthaler and Rinderle 1988). Measurement of chlorophyll fluorescence is rapid, accurate, and non-invasive (Baker 2008), and the principle of chlorophyll fluorescence analysis is relatively straightforward (Maxwell and Johnson 2000). When chlorophyll molecules in a leaf absorb light, the light

photons travel to the reaction centers of photosystems I (PSI) and II (PSII), where they can be used by photosynthesis (photochemistry), lost as heat, or re-emitted as light-chlorophyll fluorescence (Maxwell and Johnson 2000; Zhuang et al. 2020). Because each of these processes competes with the others, ChlF can be used to provide information on photosynthesis (Maxwell and Johnson 2000; Murchie and Lawson 2013). To determine the most important fluorescence kinetic parameters, such as the maximum quantum efficiency of the PSII (F_v/F_m), the polyphasic fluorescence transient is used (Zhuang et al. 2020). PSII is regarded as a susceptible component of the photosynthetic machinery and plays a central role in photoinhibition leading to a decrease in F_v/F_m (Long et al. 1994). Under stress, a decrease in F_v/F_m indicates down-regulation of photosynthesis or photoinhibition (Lichtenthaler and Rinderle 1988).

Despite considerable progress in understanding the effects of drought stress on photosynthesis and the responses of aboveground tree components to drought, little is known about how below-ground tree components respond to drought and the role roots play under drought stress. This aspect of climate change research is frequently neglected or completely ignored and further research is required (Brunner et al. 2015). However, tree root systems, in contrast to herbaceous plant root systems, are more complex, varying in morphology, size, and function (Brunner et al. 2015; Mariën et al. 2021). Coarse roots, for example, with a diameter of more than 2 mm, are responsible for tree stability and act as organs for transporting water from deeper soil horizons. Fine roots (< 2 mm in diameter), which are often non-woody and short lived, are important for the uptake of both water and nutrients (Mariën et al. 2021). In addition, roots can serve as sensors of water deficit and send signals to aboveground shoots (Hamanishi and Campbell 2011). In general, roots are typically described by several different traits such as length (cm), surface area, diameter, root volume, or lifespan of the root tips. Evidence continues to increase that drought can influence the structure and growth of tree roots (Kozłowski and Pallardy 2002).

In this study, we consider drought stress as a main driver of singular importance to ecological change and species distribution shifts. To this end, we conducted a greenhouse experiment with seedlings, as the most vulnerable stage in the tree ontogeny (juvenile stage responses), of four forest-forming tree species for the temperate climate of Central Europe, including two conifers, *Pinus sylvestris* (L.) and *Picea abies* ((L.) H. Karst), and two broadleaved deciduous species, *Fagus sylvatica* (L.) and *Quercus robur* (L.). We investigated how these four species responded to drought stress and duration at both the physiological and morphological level. We paid particular attention to chlorophyll fluorescence measurements to track changes in photosynthetic efficiency (photoinhibition) of the selected species under

drought stress and the duration of exposure to this stressor while using tree aboveground growth and root responses to drought as the morphological indicators of stress. Our focal species differ in their ecological requirements. In short, *P. sylvestris* (PS) is the most widespread Eurasian conifer. It is an early- successional pioneer tree species, light-demanding, and xerophyte tree; while, *P. abies* (PA) is a late-successional conifer species that is considered drought sensitive (hygrophyte). Both deciduous species, *Q. robur* (QR) and *F. sylvatica* (FS), are late-successional species. FS is considered a shade tolerant and mezophytic species, while QR is less shade tolerant and is considered a water demanding species (hygrophyte). Although the selected species differ in their soil moisture requirements, recent studies on species distribution models for Central Europe under expected climate change scenarios consider both conifer species as ‘losers’, while the late-successional broadleaf species QR and FS are among the ‘winner’ species (Dyderski et al. 2018). Because selection of the best species is critical to the sustainability of managed forests in a rapidly changing climate (Saxe et al. 2001), our study contributes to the broader discussion regarding species drought resistance during the vulnerable juvenile in the face of climate change.

Materials and methods

Plant material and experimental design

Seeds of all four species used in this study were collected from autochthonous stands in Poland. Seeds of the deciduous tree species were sown in spring 2020 at a local forest nursery into 265 ml Hiko containers with 28 cavities (Stuewe and Sons., Inc., USA). In the first year of growth, the seedlings were grown in a commercial nursery under operational conditions. The conifer seeds were sown at the end of April 2021 into 120 ml Hiko containers with 40 cavities (Stuewe and Sons., Inc., USA). Each container cavity was filled with a substrate consisting of peat (83–85%) and perlite (12–15%) deacidified with dolomite (pH = 6.5), and a slow-release fertilizer was added at 3 g l^{-1} (Osmocote Exact Standard 3–4 months N:P:K:Mg 15:9:12:2, with micronutrients). All containers were transported to the

research greenhouse in May 2021 and maintained in a controlled environment. During germination and in the initial phase of growth (until June), all seedlings in the containers were irrigated every day up to field capacity. Volumetric soil water content (VWC) was maintained at field capacity ($0.25\text{--}0.30 \text{ m}^3 \text{ m}^{-3}$). To compare species-specific responses to treatment, we applied the same irrigation regime for each species.

Experimental design and treatment

The experiment was established in the research greenhouse at the Forest Research Institute, Sękocin Stary, Poland, on June 28, 2021. All containers were arranged in a full factorial, split-plot design with species (PS, PA, QR, FS) and treatments: control (well watered) and drought as factors. The experiment was comprised of a total of 732 seedlings (Table 1). The containers with seedlings were divided into two groups. One group of seedlings ($n = 352$) was subjected to drought by withholding irrigation (the irrigation lines were closed), while the remaining seedlings ($n = 380$) were irrigated, maintaining the volumetric soil water content at $\sim 0.25\text{--}0.30 \text{ m}^3 \text{ m}^{-3}$ for the duration of the experiment, which ran for over 60 days. The soil temperature and volumetric water content were monitored using Decagon EM50 data loggers with 5TM soil sensors (Decagon Devices, Pullman WA, USA). The mean soil moisture at the beginning of the experiment was 25.3% and decreased to 0.0% at the end of the experiment in the drought treatment. The soil moisture and soil temperature monitoring across experimental treatments and time are presented in Electronic supplementary material (Fig. 1S and Fig. 2S). During the study period, containers were re-randomized within treatments every 10 days to avoid bias in growth conditions and edge effects. Monitoring of height growth and maximum quantum yield of PSII on selected seedlings was treated as repeat measurements.

Physiological responses of seedlings to drought—fluorescence chlorophyll

The maximum quantum yield of PSII (F_v/F_m , where $F_v = F_m - F_0$ represents variable fluorescence in the dark-adapted state, F_m represents maximum fluorescence in the

Table 1 Species, seedlot code, number (No.) of seedlings, container type, and number of containers used in the greenhouse drought experiment

Species	Certificate of forest reproductive material (seedlot code)	No. of seedlings	Container type	No. of containers
<i>Pinus sylvestris</i>	MR/58600/20/PL MR/51864/17/PL	320	Hiko HV120SS (40 cavities)	8
<i>Picea abies</i>	MR/18209/08/PL	160	Hiko HV120SS (40 cavities)	4
<i>Fagus sylvatica</i>	MR/59570/19/PL	112	Hiko V265 (28 cavities)	4
<i>Quercus robur</i>	MR/53589/17/PL	140	Hiko V265 (28 cavities)	5

dark-adapted state, and F_0 represents minimum fluorescence in the dark-adapted state (Mena-Petite et al. 2000) was measured seven times during the experiment at 10-day intervals beginning on 28 June (Day 1 of the experiment) on fully watered seedlings. The last measurements were performed on August 30 (~Day 60 of drought).

Five randomly selected seedlings from each conifer species per container and four randomly selected seedlings from each deciduous species per container, in each treatment (total 96 seedlings), were permanently marked for repeated measurements. F_v/F_m measurements were performed on the uppermost whorl of the conifer seedlings and on the uppermost expanded leaf of the deciduous seedlings. Prior to measurement, needles and leaves were placed in a clip and allowed to adjust to darkness for a minimum of 30 min. Minimum fluorescence (F_0) was measured for the dark-adapted photosynthetic apparatus, and maximum fluorescence (F_m) was measured after a 0.7-s saturating light pulse. The maximum variable fluorescence ($F_v = F_m - F_0$) and the maximum quantum yield of PSII (F_v/F_m) were calculated automatically. Chlorophyll a fluorescence was evaluated with the Plant Efficiency Analyser (PEA, Hansatech, Norfolk, UK).

Morphological responses of seedlings to drought—height and root architecture

Height was measured three times during the experiment with a ruler from the surface of the soil to the tip of the shoot. The first measurement was taken four days before the start of the experiment (24.06.2021), the second measurement at 31 days after the start of the experiment (28.07.2022), and the last one on the last day of the experiment (30.08.2021).

At the end of the growing season (October), 42 seedlings were randomly selected and harvested to evaluate the root system parameters of the drought-stressed and well-watered seedlings. Immediately after harvest, roots were marked and kept in a cooler (5 °C) prior to being soaked in water several times to remove the substrate. Rinse water was poured through sieves to collect any roots that detached during rinsing. The cleaned root systems were wrapped in a paper towel and placed in foil bags in the 5 °C cooler until measurements were taken.

Root systems were scanned for eight characteristics (total root length, total root area, average root thickness, root system volume, number of root tips, number of root forks, number of root crossings, and number of thickness classes) in a container filled with water using an Epson Perfection V800 / V850 scanner (software version 1.9 V3.93 3.9.3.4) adapted to work with the WinRHIZO software (Regent Instrument version 2017a). The scan was performed at a resolution of 600 dpi in 16-bit grayscale which made it possible to clearly

separate the root system from the background. The image was saved in TIFF format.

Statistical analysis

F_v/F_m was plotted on heat maps as a function of soil VWC and temperature for each species, using a spline function for smoothing the data. To evaluate the differences in seedling height (measured three times) and the maximum photochemical efficiency of PS II (measured seven times), we used a general linear model for repeated measures. The partial Eta squared (η_p^2) was used to describe the effect sizes. Partial eta squared (η_p^2) was expressed in per cent as the sum of squares of the effect (SS_{effect}) in relation to the sum of squares of the effect and the sum of squares of the error associated with the effect (SS_{error}) (Lakens 2013).

Contrast analysis was performed to further explore the dataset which allowed testing the statistical significance of the predicted detailed differences in specific parts of the complex experimental system (Stanisz and Tadeusiewicz 2007). Contrasts between the drought treatment and the control were determined separately for each species by measurement dates. This approach made it possible to follow when seedlings of a given species in the drought treatment began to deviate in parameters from the control seedlings. Specifically, we tested the hypotheses $H_0: \mu_1 - \mu_2 = 0$ vs $H_1: \mu_1 - \mu_2 \neq 0$; where μ_1 represents the mean for a given species in the well-watered treatment at a specific time point and μ_2 represents the mean for the same species in the same time point in the drought condition, which were verified by the significance of the contrast vector (L). The required vector with contrast weights was obtained directly from these hypotheses: $L = [-1 \ 1]$. The statistical significance of L was verified by the F-test and the calculated probability (p-value) of a value of an F (Haans 2018).

For the eight root traits measured at harvest, multivariate analysis of variance (MANOVA) was used. This analysis was followed by ANOVA tests for each root trait separately. An assessment of experimental effect sizes was performed using η^2 . Eta squared (η^2) was expressed in per cent as the sum of squares of the effect (SS_{effect}) in relation to the total sum of squares (Lakens 2013). Root traits were also subjected to contrast analysis with contrasts defined for the treatments (drought and control) separately for each species. The null hypothesis was rejected if this probability was less than or equal to 0.05 for all measured characteristics. All statistical analyses were performed using the Statistica 13.0 statistical package (TIBCO Software Inc. 2017).

Results

Maximum quantum yield of PS II

The trends in the maximum efficiency of PSII as a function of soil VWC and temperature were nonlinear and species specific (Fig. 1). The photosynthetic capacity of

PSII was largely dependent on water availability and only slightly dependent on temperature. With the exception of QR (Fig. 1b), which was sensitive to lower temperatures, all species exhibited a wide range of thermal optimum for maximum PSII efficiency. For this reason, temperature was removed from further analyses.

Seedlings of all species were able to maintain relatively high F_v/F_m values at optimal temperature, even under severe

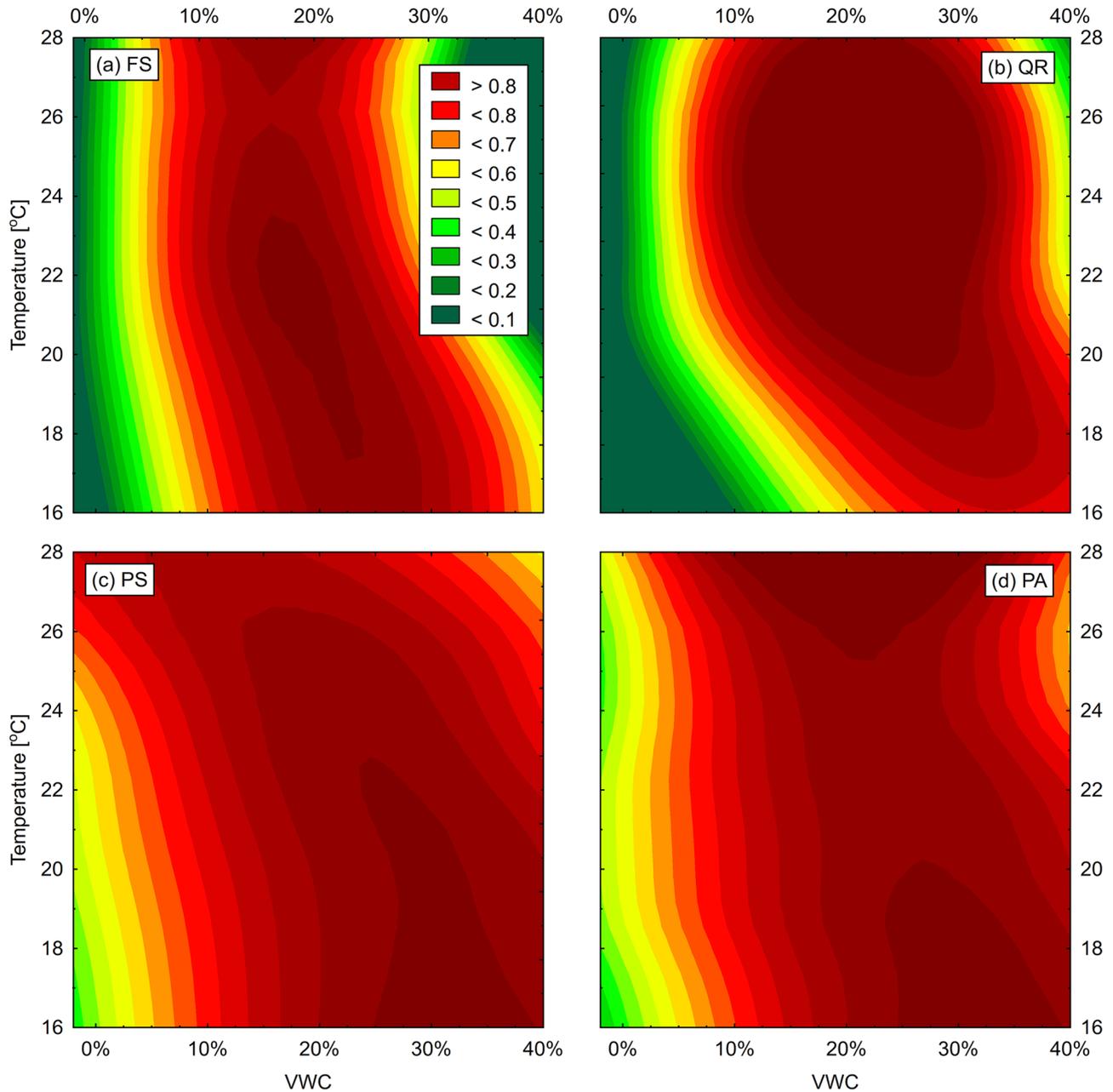


Fig. 1 Heat maps showing variation in F_v/F_m as a spline function of soil volumetric water content (VWC) and temperature for *Fagus sylvatica* (a), *Quercus robur* (b), *Pinus sylvestris* (c), and *Picea abies* (d). Predicted values of species-specific maximal photosynthetic

capacity of PSII are shown as smooth surfaces representing pooled F_v/F_m data (for all terms and both treatments). F_v/F_m is scaled to a color gradient ranging from low (green) to high (red)

Table 2 ANOVA with repeated measurements for the maximum photochemical yield of photosystem II (F_v/F_m) of seedlings over time and experimental effects: species and treatment (control vs. drought) including sums of squares (SS), degrees of freedom (Df), mean squares (MS), f-value (F), p-value (p), and $\eta_p^2\%$

Effects	SS	Df	MS	F	p	$\eta_p^2\%$
Treatment	11.5226	1	11.5226	168.745	<0.001	42
Species	4.3838	3	1.4613	21.400	<0.001	65
Treatment×Species	2.9704	3	0.9901	14.500	<0.001	33
Err	6.0090	88	0.0683			
Time	10.2726	6	1.7121	77.903	<0.001	46
Time×Treatment	8.8276	6	1.4713	66.945	<0.001	24
Time×Species	3.8534	18	0.2141	9.741	<0.001	43
Time × Treatment × Species	2.6569	18	0.1476	6.716	<0.001	18
Err	11.6041	528	0.0220			

water stress (less than 10% VWC). However, the deciduous and coniferous species showed different behavioral patterns to cope with water deficit. The deciduous species responded rapidly with photoinhibition in a very narrow range on the VWC axis when VWC dropped below 10%. In contrast, maximum PSII efficiency gradually decreased in PA and PS under increasing water scarcity (Fig. 1).

Leaf F_v/F_m showed significant variation between all main effects: treatment, species, time, and interaction effects (Table 2). The contrast analysis allowed us to track species-specific changes in F_v/F_m with drought stress over time. The contrast analysis showed F_v/F_m decreased significantly in the drought treatment in QR at Day 20 ($p=0.003$) of the experiment compared to well-watered seedlings. After Day

30, significant differences (drought vs. well watered) were observed in the efficiency of the PSII in FS ($p<0.001$). PA lost photosynthetic capacity after Day 40 compared to the well-watered seedlings ($p=0.043$), while PS significantly decreased its photosynthetic capacity after Day 50 ($p=0.001$; Fig. 2). Importantly, when we considered the detailed results of species-specific VWC status over time, we found that broadleaved deciduous and coniferous species differed in terms of their soil water conservation strategy. QR and FS depleted available water resources to 10% (VWC) very quickly, in 11 and 18 days, respectively; while the conifers, PS and PA, maintained higher VWC (above 10%) over a longer period, 42 and 47 days, respectively (ESM, Fig. 1S).

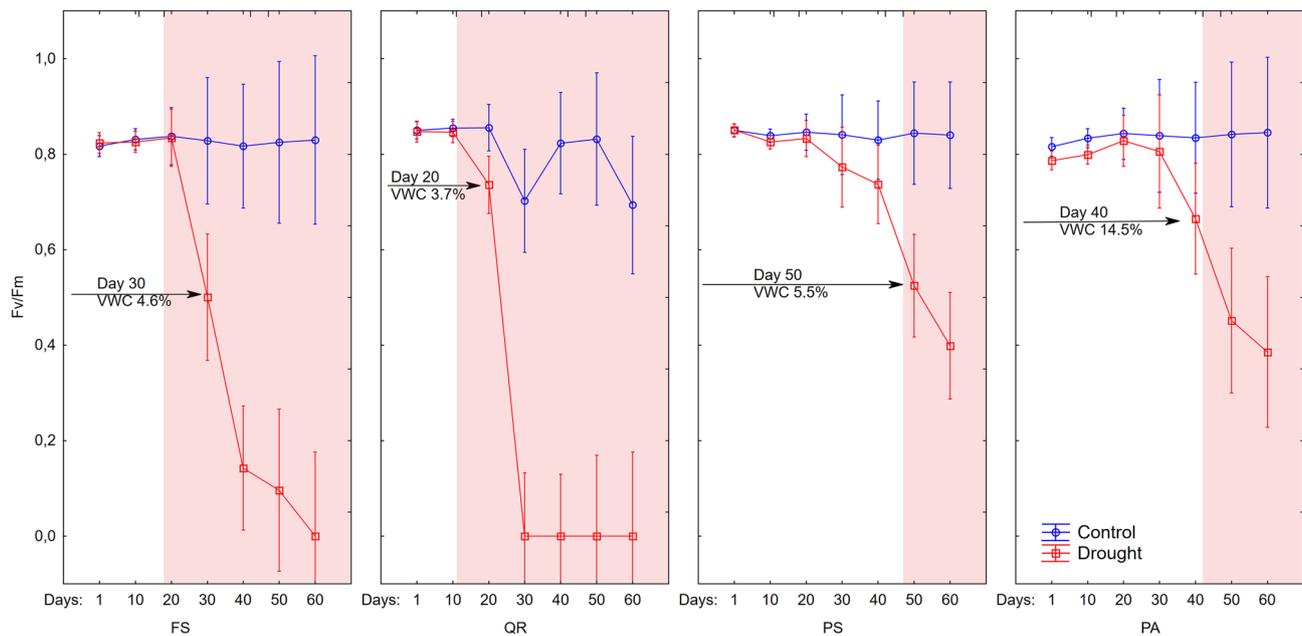


Fig. 2 Maximum photosynthetic efficiency of photosystem II (F_v/F_m) in seedlings from four tested species: *Fagus sylvatica* (FS), *Quercus robur* (QR), *Pinus sylvestris* (PS), and *Picea abies* (PA) over time, taking into account treatment (drought and control (irrigation)). The horizontal arrows mark the first measurement date from which sta-

tistically significant contrasts between the F_v/F_m of seedlings in the control and drought treatments occurred. The semi-transparent red area for each species denotes the period of severe water stress for the seedlings and begins on the day when the VWC fell below 10%

However, when the effect of VWC and sensitivity of PSII to drought were combined, the species ranking changed considerably (Fig. 2). Here, PA was the most sensitive species, responding to water scarcity with photoinhibition when soil VWC dropped to 14.5%. The second most sensitive species was PS, whose F_v/F_m decreased significantly compared to well-watered seedlings at a VWC status of 5.5%. The broadleaved deciduous species showed photoinhibition only at critically low VWC (below 5% VWC), which was particularly evident in QR (3.7%, Fig. 2).

Height

The analysis of variance for repeated measurements revealed significant differences in height among species, over time, and in the interaction between time and species. QR had the highest recorded height, followed by FS, while both conifer species were considerably smaller. The seedlings' height changed significantly over time, which was related to the natural elongation process during the active growth phase. This height variation exhibited species-specific growth patterns. Specifically, the growth of the broadleaved species plateaued midway through the experiment, while conifer species exhibited a continuous height increment throughout the entire study period. However, the species-specific height growth trajectories remained consistent across both control and drought treatment groups (Table 3; Fig. 3).

Responses of root traits to drought

Multivariate analysis of variance (MANOVA) for root traits showed differences in tree responses depending on the treatment (control vs. drought) and tree species. The interaction between tree species and experimental treatment was also significant, indicating that specific root responses to drought were also depend on tree species (Table 4).

Results from ANOVA, conducted separately for each root trait (Table 5), showed that species-specific responses explained the greatest part of the variability in the model (48–60% of the variability according to the Eta squared coefficients, excluding the number of root thickness classes), while treatment (control vs. drought) explained another 6–12% of the variability described in the model (Table 5). The interaction between species and treatment was not significant for most root traits (except for mean diameter), and explained the smallest part of the variability in the model (values of the Eta-Squared coefficients ranged from 4 to 8%).

Results of the contrast analysis delineate that the tree responses to drought stress, as manifest in root morphological traits, can be categorized into species-specific and, on a broader scale, broadleaved deciduous and coniferous species groupings (Fig. 4). The deciduous species were sensitive to drought stress, particularly FS, which exhibited reduced root

length, thickness, volume, and surface area under drought conditions and produced significantly fewer forks, tips, and crossings than roots of seedlings of this species under the well-watered control conditions. QR, was the second most sensitive species to drought. Coniferous species appeared less sensitive to the concomitant phenomenon of drought, although the PA seedlings produced significantly fewer root tips under drought conditions than under well-watered conditions (Fig. 4).

Discussion

In this study, we investigated the maximum quantum efficiency of PSII (F_v/F_m), height growth, and root morphology during soil drying in four woody species selected as representative of the major forest-forming tree species in Central Europe. Our focal species showed different strategies to cope with water limitation, including morphological and physiological changes. The two deciduous tree species (FS and QR) were characterized by significantly higher soil water uptake than the conifer species (PS and PA), which was related to their faster growth rate in the juvenile stage as an adaptation strategy to successfully compete with other trees, shrubs, and herbaceous plants for soil and light resources in nature. As a result, they quickly consumed the available water resources in the limited space of the nursery container, experiencing severe drought soon after the experiment began. This was particularly evident in QR, the tallest of the four species investigated (11 days in QR and 18 days in FS).

Leaf F_v/F_m appeared to be an appropriate and sensitive probe for the rapid assessment of physiological stress levels in seedlings (Meng et al. 2016; Zhuang et al. 2020). F_v/F_m decreased significantly under drought stress, indicating that PSII was damaged under drought stress and the primary response of photosynthesis was inhibited, which is consistent with Lichtenthaler and Rinderle (1988), and Meng et al. (2016). In addition, the leaf F_v/F_m response of seedlings to drought was species-specific and dependent on drought duration, indicating differential drought susceptibility among the species studied.

Overall, the physiological patterns of response differed between the deciduous and conifer species (Figs. 1 and 2). While both conifers conserved soil water resources over a longer period of time, and thus maintained higher F_v/F_m values for a longer time period than the deciduous trees, they showed greater sensitivity to drought-induced photoinhibition at relatively higher soil moisture (5–15% VWC) than the deciduous species. The gradual decline in maximum quantum efficiency of PSII, in response to decreasing VWC in both coniferous species (Figs. 1c and d) indicated their sensitivity to soil moisture fluctuations, reflecting their

Table 3 ANOVA with repeated measurements for seedling height over time and experimental effects: species and treatment (control vs. drought)

Effects	SS	Df	MS	F	p-value	$\eta_p^{2\%}$
Treatment	8078	1	8078	0.775	0.379	< 0.1
Species	15347160	3	5115720	490.770	< 0.001	68
Treatment×Species	92319	3	30773	2.952	0.032	1
Error	7015264	673	10424			
Time	75539	2	37770	315.480	< 0.001	31
Time×Treatment	153	2	77	0.640	0.527	0
Time×Species	37040	6	6173	51.564	< 0.001	18
Time × Treatment × Species	881	6	147	1.226	0.230	< 0.1
Error	161145	1346	120			

isohydric water management strategy. This increased sensitivity to water scarcity was particularly striking in PA, which showed photoinhibition at a VWC of 14.5% (moderate water stress; Fig. 2). In contrast, deciduous tree seedlings depleted soil water quickly, but reduced F_v/F_m only under the adverse conditions of severe drought (VWC < 5%; Fig. 1a and 1b; Fig. 2), operating within a narrow safety margin, characteristic of anisohydric behavior. This behavior was particularly pronounced in QR, which showed photoinhibition at a critically low VWC level of 3.7% (see Fig. 2). Méthy et al. (1996) and Epron and Dreyer (1993) also observed low sensitivity of F_v/F_m to moderate drought stress in several European *Quercus* species, and F_v/F_m decreased only under severe water stress (at predawn water potentials below -4 MPa). Accordingly, the rapid soil water consumption of

this species in our study could be attributed to its maintenance of high transpiration, stomatal conductance, and photosynthesis under moderate drought conditions (Epron and Dreyer, 1993; Aranda et al. 2000), while photoinhibition occurred within a narrow safety margin under severe stress. FS similarly displayed anisohydric behavior, albeit conserving water slightly longer than QR (Fig. 2, Fig S1), implying a lower water use in this species. The variation in F_v/F_m values, shifting from optimal conditions (F_v/F_m above 0.8) to adverse conditions (F_v/F_m close to 0), exhibited a broader safety margin dependent on VWC in FS compared to QR (cf. Figures 1a and 1b). These results may indicate that FS exhibits transitional behavior on the anisohydric–isohydric scale. Indeed, FS is frequently described as aniso- or isohydric, which, as Nguyen et al. (2017) noted, depends on site

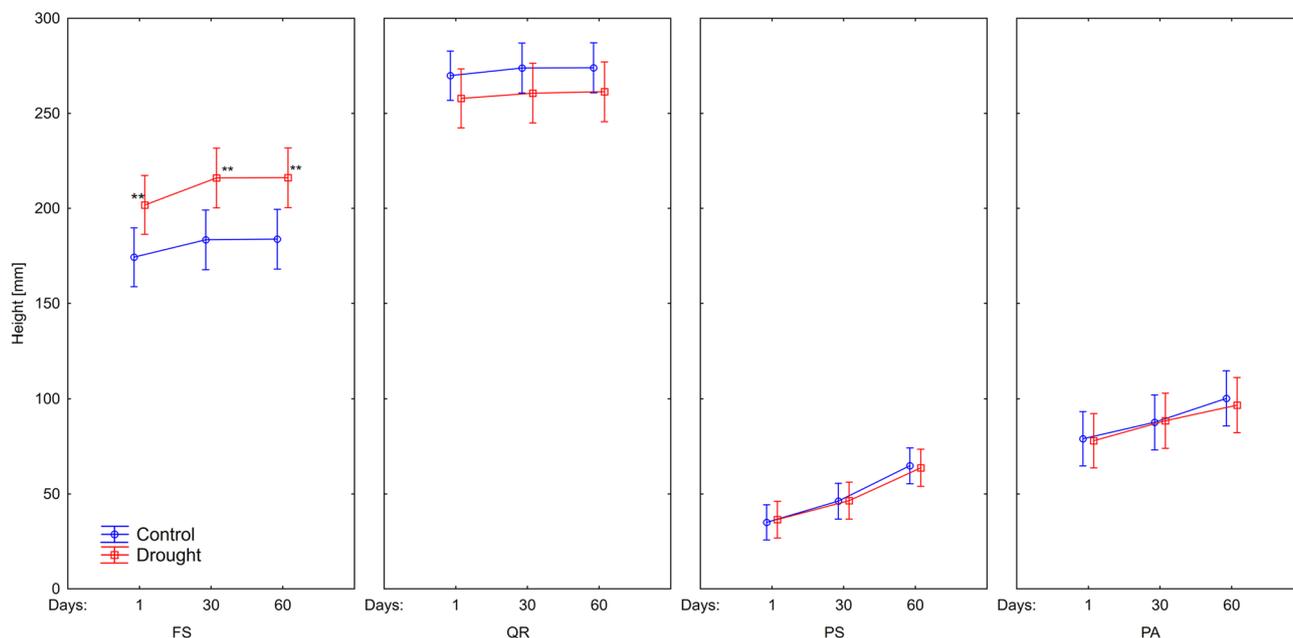


Fig. 3 Mean seedling height (\pm SE) of the four studied species: *Fagus sylvatica* (FS), *Quercus robur* (QR), *Pinus sylvestris* (PS), and *Picea abies* (PA). Statistically significant contrast between the height

of seedlings in the drought vs. control treatments marked by ** at $p \leq 0.05$ for a given measurement date

Table 4 MANOVA for eight root traits depending on species and experimental treatment (drought vs. control)

Wilk Test	F	Factor Df	Err Df	p-value
0.005	16.574	24	78.909	<0.001
0.528	3.016	8	27.000	0.015
0.079	4.606	24	78.909	<0.001

conditions and provenance, thus showing large intraspecific variation in this species.

Our analysis of height growth measurements revealed no differences in shoot elongation between treatments, although a reduction in growth due to a decrease in cell expansion is typically the first morphological and quantifiable effect of drought stress (Kramer 1974; Szym-Borowska et al. 2022). The lack of significant differences in height between irrigated and non-irrigated seedlings may be primarily because our study focused on the effects of summer drought (July–August). The phenology of FS and QR, indicates that both species typically complete shoot elongation and bud formation for the following year in June in growing conditions in Poland (before the start of our experiment) (Chałupka 1990, 2006). Despite the fact that both FS and QR are capable of producing proleptic shoots in secondary growth in July (proleptic shoots typically make up 5% of all shoots in a given year) (Chałupka 1990, 2006), neither species demonstrated this behavior in the current study, even under the well-watered control treatment. In turn, the seedlings of the coniferous species, characterized by a longer elongation period during the growing season (they usually complete shoot elongation in July) (Chałupka 1977, 1993), were able to complete their height growth before drought conditions came into effect, mainly due to a more conservative management of water resources. The PS and PA species did not experience the summer drought until 42 and 47 days after the start of the experiment (August), respectively. Thus, the absence of summer drought effects on height growth in all species studied can be explained by the earlier timing of shoot elongation and bud formation phenology (especially in FS and QR) and by conservative strategies to cope with water limitation (especially in PS and PA). These results are consistent with Taeger et al. (2015) but do not rule out negative consequences in subsequent years (Thiel et al. 2012).

Contrary to the aboveground growth, the below-ground root systems of the deciduous seedlings were sensitive and responsive morphological probes of drought severity. The deciduous tree species exhibited a sensitive morphological response to soil drying, indicating that they experienced severe drought stress. The results of meta-analysis of several studies have shown that root growth response is strongly influenced by the severity of stress (Poorter et al. 2012). Plants exposed to a moderate water deficit generally

Table 5 ANOVA for individual root traits for experimental effects: species and treatment (control vs. drought). η^2 —the proportion of the total variance explained by the experimental effect

Effect	DF	Length	Surface area		Average diameter		Root volume		Tips		Forks		Crossings		Number of diameter classes	
			F (p-value)	η^2 [%]	F (p-value)	η^2 [%]	F (p-value)	η^2 [%]	F (p-value)	η^2 [%]	F (p-value)	η^2 [%]	F (p-value)	η^2 [%]	F (p-value)	η^2 [%]
Species	3	17.516 (<0.001)	24.65 (<0.001)	56	32.433 (<0.001)	60	26.012 (<0.001)	57	16.758 (<0.001)	48	21.262 (<0.001)	55	19.854 (<0.001)	56	1.247 (0.308)	8
Treatment	1	10.578 (0.003)	10.058 (0.003)	7	4.753 (0.036)	2	8.555 (0.006)	6	12.92 (0.001)	12	8.648 (0.006)	7	6.822 (0.013)	6	3.332 (0.077)	7
Species × Treatment	3	1.855 (0.156)	2.556 (0.071)	5	4.548 (0.009)	8	2.615 (0.067)	5	2.087 (0.12)	6	2.447 (0.081)	6	1.518 (0.227)	4	1.247 (0.308)	8

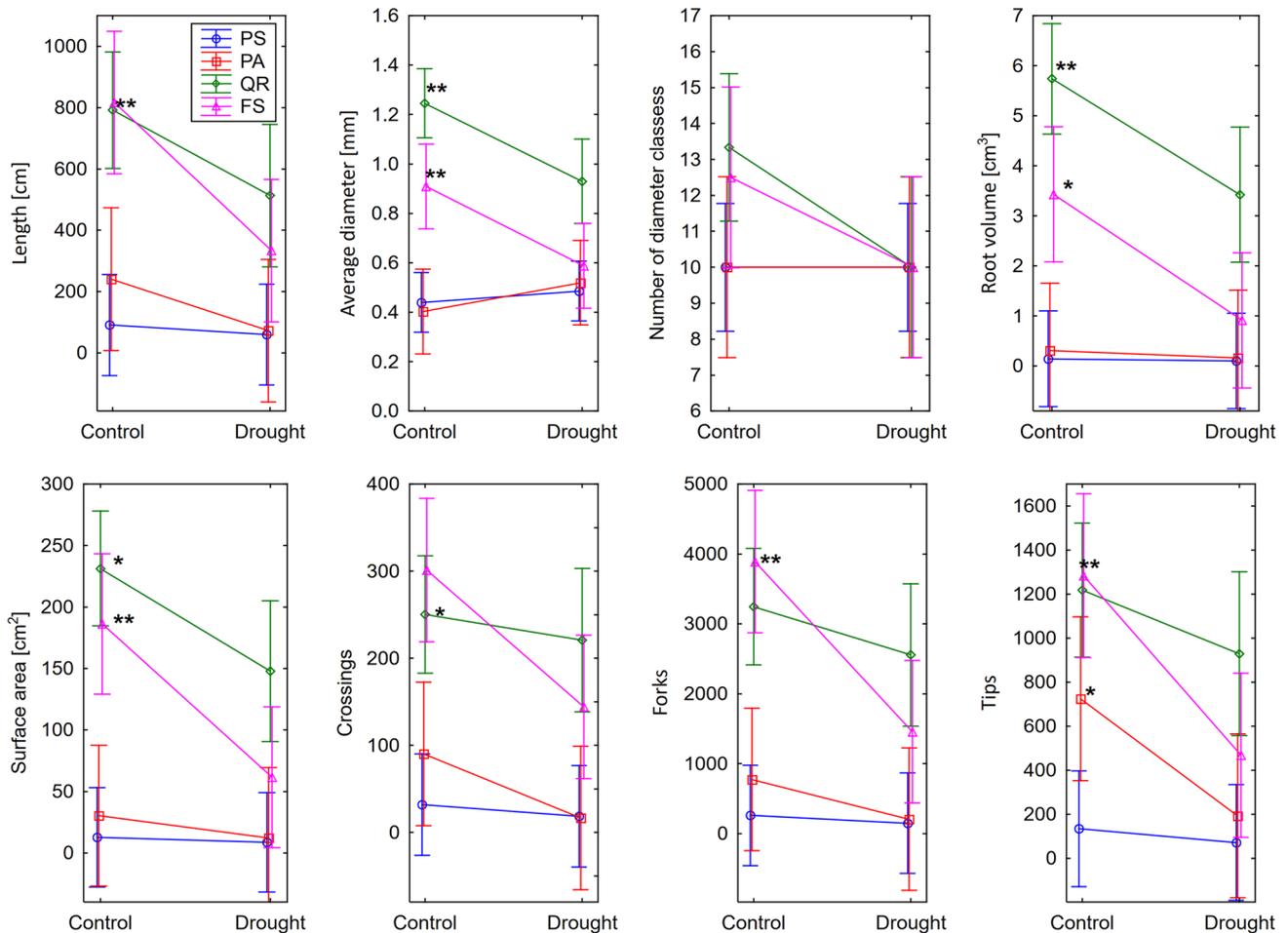


Fig. 4 Means (\pm SE) for eight root characteristics measured after the end of the experiment, showing the contrast analysis between the drought vs. control morphological traits separately for each species. Markings: *—significant contrast at the level of $p \leq 0.05$, **—signifi-

cant contrast at the level of significance of $p < 0.01$. *Fagus sylvatica* (FS), *Quercus robur* (QR), *Pinus sylvestris* (PS), and *Picea abies* (PA)

show little change in their growth pattern, with only a small increase in root biomass relative to total biomass, maintaining aboveground growth and thus their competitiveness for aboveground resources for as long as possible. In contrast, plants exposed to severe drought typically increase the proportion of root mass, with a concomitant reduction in stem growth (Brunner et al. 2015). However, despite an increase in root-to-shoot ratio during drought, fine root biomass, root length, and root tip frequency are typically reduced (Brunner and Godbold 2007; Eldhuset et al. 2013; Zang et al. 2014; Brunner et al. 2015). These results are consistent with our observations made on deciduous tree species under drought stress, particularly in FS, which, based on contrast analysis, significantly reduced root length, volume, area, thickness, root tip frequency, forks, and crossings in the drought treatment compared with the control. Interestingly, FS was characterized by a greater or comparable number of crossings, forks, and root tip frequency, traits important for water

uptake, in the control treatment compared to QR, although the latter species was much taller (aboveground) suggesting that FS might outperform QR in mixed stands under normal or moderate drought stress conditions. This finding is consistent with previous studies indicating that QR (and *Q. petraea*) have a lower competitive ability than FS in sites with moderate or optimal water and mineral supplies (Kieliszewska-Rokicka 2006). As opposed to the QR and FS species, root traits of the conifers were largely insensitive to soil water scarcity, with only a very weak decrease in root tip frequency in PA under drought treatment. This result is probably due to a more conservative use of soil water resources in the juvenile stage, which allowed the conifers to maintain a higher VWC value for a longer period during the growing season, thus avoiding a significant loss in root trait parameters.

Although we maintained uniform soil conditions in our experiment, it is crucial to acknowledge that our focal

species inhabit diverse soils in nature in terms of fertility and texture. The two deciduous species require deep, fine-textured soils, while the conifers often grow in sandy, coarse-textured soils, that are expected to experience greater hydraulic failure during drought (McDowell et al. 2008). The depth of the water table also affects plant hydraulics by limiting or allowing plant water uptake during drought periods (Dawson 1996; Franks et al. 2007). To compensate for coarse-textured soils, or inaccessible water tables, trees can increase their soil root or rhizosphere conductance by adjusting their fine root density (Ewers et al. 2000; Hacke et al. 2000), fine root hydraulic conductance (McElrone et al. 2007), root depth, and other root characteristics (Stirzaker and Passioura 1996). However, our results suggest that this adjustment may occur under moderate drought stress rather than severe drought, while it remains unclear, to what extent root characteristics may change depending on soil properties and water table depth in nature.

Conclusions

In conclusion, our study highlights distinctly different soil water use strategies and trade-offs during the juvenile growth stage of our four focal species. Conifers adopted a water-conserving strategy, while the deciduous species employed efficient water usage for photosynthesis and growth, resulting in more rapid soil water depletion. These responses also reflect different drought tolerance and avoidance behaviors. The two deciduous tree species (FS and QR) exhibited similar anisohydric responses to drought stress, indicating a degree of drought tolerance. In contrast, ecologically differentiated conifers such as PS and PA demonstrated isohydric behavior in response to water scarcity during their juvenile growth phase, indicating a water stress avoidance strategy. It is worth noting that species responses to drought and its duration were more nuanced than these general water management strategies suggest. Although PA and PS exhibited similar patterns of water management, photoinhibition in these species occurred at very different VWC levels, 14.5% and 5.5%, respectively, demonstrating a wide range of species-specific responses to water scarcity.

Considering water stress tolerance, the anisohydric deciduous tree species seem to be better candidates for forest management under climate change. On the other hand, PS with its water-conserving management and relatively low threshold for photoinhibition response (5.5% VWC), in addition to its minimal morphological responses to drought stress during the juvenile stage, promises to be a good alternative for successful regeneration in drought-prone forest areas under future climate conditions. However, it is unclear to what extent the current results on container nursery seedlings can

be extrapolated to older growth stages and growth under natural conditions. Thus, further research is needed to fill these knowledge gaps.

In addition to photochemical responses to drought, which have been shown to be accurate indicators of physiological changes, we also observed distinct morphological responses to water scarcity in the species studied, particularly in root characteristics, suggesting that broadleaved species are more sensitive. Nevertheless, our study does not provide insight into the extent to which our focal species can make site-specific adaptations (e.g., root architecture) or whether they alter their behavioral patterns to adjust to different site conditions, which seems plausible given the large intraspecific variation reported among species. Consequently, the insights gained in this study contribute to an increase in our understanding of species-specific responses to drought stress. The results also provide a solid foundation for further studies that consider these nuanced strategies and responses in a broader ecological context, aiming to improve forest management approaches under changing environmental conditions.

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Author contributions Conceptualization: MN; Formal analysis: MN; Funding acquisition: MN; Investigation: MN and SJ; Methodology: MN; Writing—original draft preparation: MN; Review and editing: MN and BRT. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets generated and/or analyzed during the current study can be available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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