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Research paper

Drought memory expression varies across ecologically contrasting forest tree species

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ABSTRACT

Trees may exhibit long-lasting morpho-physiological acclimation in response to drought (i.e. drought memory) throughout their extensive life cycles. This acclimation might be particularly crucial for seedlings and saplings due to their limited access to water. We studied the development of drought stress memory in seedlings of black walnut (*Juglans nigra L.*), western larch (*Larix occidentalis* Nutt.), and Douglas-fir ((*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) in response to controlled drought exposure during their germination year (drought priming). We evaluated the effects of drought priming under a second-year drought, focusing on changes in water uptake capacity and transpiration demand, biomass allocation to new roots and foliage, root architecture, and photosynthesis. Drought priming led to significant morpho-physiological responses in the new leaves and roots developed during the subsequent growing season drought. Western larch showed increased biomass allocation to roots, higher specific root length and root tips, and enhanced water uptake, while Douglas-fir exhibited earlier bud break, greater net photosynthesis, and increased foliage growth. In contrast, black walnut seedlings displayed no notable changes in biomass allocation or physiology. Our results also show that biomass allocation to new roots plays a crucial role in enhancing water uptake capacity and gas exchange during seedling establishment. These findings underscore the importance of drought memory for stress resistance in trees, influencing the capacity of forests to regenerate and respond to recurrent droughts and climate change. The formation and expression of drought memory, however, varied across species, highlighting the complexity of adaptive responses across different forest ecosystems.

1. Introduction

As climate change intensifies droughts in many forested areas worldwide, the ability of trees to acclimate to recurrent drought exposure becomes critical for forest regeneration (Schwalm et al., 2017). Central to this adaptation could be drought stress memory, wherein trees retain information from past drought stress (drought priming), leading to an enhanced responses in subsequent droughts or sustained response following drought priming (Galviz et al., 2020; Lämke and Bäurle, 2017; Walter et al., 2013). From an epigenetic perspective, these memory mechanisms are categorized as: I) Type 1 memory (or sustained induction), which enables plants to maintain stress resistance through sustained high levels of resistance gene expression even after the initial stress has subsided; and II) Type 2 memory (modified re-induction), which alters the expression genes, resulting in stronger and faster reactivation of these genes upon subsequent stress exposure (Lämke

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et al., 2016; Liu et al., 2022; Charng et al., 2023; Crawford et al., 2024). This phenomenon introduces a temporal dimension to acclimation (memory driven acclimation), allowing plants to leverage past stress alongside current conditions to maintain or enhance resistance to recurrent stress. Stress memory can last from days, years, and, in some cases, across generations (Fossdal et al., 2024; Lämke and Bäurle, 2017; Skrøppa et al., 2007; Yakovlev et al., 2012). Drought memory has been documented in herbaceous plants and crops (Galviz et al., 2020; Jacques et al., 2021; Tombesi et al., 2018; Wojtyla et al., 2020). However, despite trees generally having long lifespans and therefore potentially depending on phenotypic plasticity for survival, research on the presence and importance of memory driven plasticity and specifically stress acclimation is scarce. This temporal dimension in the relationship between stress and phenotypic plasticity is especially relevant in early life stages when tree mortality is most likely to occur. Evidence of drought stress memory has been observed in tree-ring data (Mu et al., 2022), and during successive short drought events within one growing season (Clemens and Jones, 1978; Guarnaschelli et al., 2006), but the importance of drought memory in morpho-physiological acclimation across growing seasons remains unclear.

Drought stress results from an imbalance between water availability and the transpiration requirements that can trigger short- and long-term cascading effects on plants physiology and morphology. Shortcomings in the access to water under a high evaporative demand can result in a feedback cycle where growth is limited by a low gas exchange rate (Reich et al., 1998; Tomasella et al., 2017). This can result in further long-term limitations to transpiration and photosynthesis by limiting water uptake via non-functional xylem and stunted root development (Schurr et al., 2006). Ultimately, prolonged drought stress can result in mortality due to carbon starvation or hydraulic failure (Mantova et al., 2022; McDowell, 2011). Although the same processes drive water uptake and transport across the entire life of trees, seedlings and saplings are more vulnerable to drought stress and hydraulic failure than mature trees because of their limited access to consistent water reserves in deep soil horizons (Simeone et al., 2019). Non-lethal levels of drought, however, have been shown to reduce drought vulnerability by promoting the development of a drought resistant phenotype. For example, Tomasella et al. (2019) found that *Fagus sylvatica* saplings exposed to two consecutive seasonal droughts had lower water consumption via reduction of foliage area and hydraulic conductivity. Guarnaschelli et al. (2006) found that previous exposure to drought increased *Eucalyptus globulus* seedling survival during a second drought event in the same growing season because of higher root to shoot ratio and maintenance of higher relative water content.

Stress memory has also been explored in the context of drought hardening of planted forest seedlings, also known as drought priming, drought conditioning or drought preconditioning. Drought priming of tree seedlings through controlled water deficit treatments in the nursery has demonstrated potential for improving seedling establishment and survival under arid conditions (Guarnaschelli et al., 2003; Luo et al., 2022; Puértolas et al., 2024; Vilagrosa et al., 2003). However, the post-planting survival of drought primed seedlings has been inconsistent. While some studies have reported higher seedling survival following drought priming (van den Driessche, 1992; Guarnaschelli et al., 2003; Shi et al., 2019), other studies found no difference compared to well-watered control seedlings (Luo et al., 2022; Royo et al., 2001; Villar-Salvador et al., 2013, 1999). The positive outcomes of drought memory in the context of seedling hardening are mostly attributed to morpho-physiological effects such as lower water demand of drought primed seedlings due to smaller comparative size and foliage area and osmotic regulation (Puértolas et al., 2024).

The inconsistencies in memory-driven morpho-physiological acclimation to drought in trees could be due to several interacting factors. The differences in the intensity of drought priming across studies can interact with species ecophysiological characteristics and drought resistance strategies influencing the formation of stress memory and extent to which and alternate phenotypes can arise in response to iterative drought exposure (i.e. phenotypic plasticity; (Kuijper and Hoyle, 2015; Vilagrosa et al., 2003). For example, in a similar study assessing the effect of consecutive droughts during the same growing season, Ribeyre et al. (2022) concluded that a drought memory was not formed in *Acer saccharum* or *Picea glauca* based on evidence that seedlings exposed to a second drought event did not exhibit faster or stronger physiological or morphological acclimation. In addition, differences in field performance and survival in response to drought priming can be difficult to determine under mild outplanting conditions that are not stressful enough to reveal differences in performance and trigger drought memory mechanisms (Royo et al., 2001). Outplanting studies also present experimental challenges to studying root system development and physiological traits related to water uptake and demand. Thus, the few studies reporting post-planting effects of drought priming on root development of trees were carried out in greenhouses where specific outplanting conditions can be simulated (Guarnaschelli et al., 2006; Pinto et al., 2023; Tomasella et al., 2019) or through root growth potential measurements where all environmental conditions are optimized (Villar-Salvador et al., 2013).

Overall, drought priming is expected to induce morpho-physiological changes in trees that balance their transpiration demand and water uptake capacity, which potentially reduces the risk of hydraulic failure during establishment amid subsequent drought (Landis et al., 1998). However, the development and expression of memory driven morpho-physiological acclimation to drought across successive seasons remains poorly understood, especially concerning early life stages when trees are the most vulnerable to drought. Understanding this phenomenon would provide insights into tree acclimation strategies and forest regeneration in the face of shifting climate patterns.

This study assesses whether tree seedlings develop drought stress memory in response to priming during germination year evaluated through the effects on seedling development under a subsequent growing season drought. Specifically, we tested whether drought memory affects the allocation of biomass towards new roots and foliage and the correlations with photosynthesis and water relations. We selected three forest species from North America: black walnut (*Juglans nigra* L.), western larch (*Larix occidentalis* Nutt.), and coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco). In addition to having contrasting geographic ranges, these species have marked ecophysiological and life history differences (Table 1; Herman et al., 1990; Schmidt et al., 1990; Williams, 1990), emphasizing their divergence within the plant economic spectrum (Reich, 2014). Black walnut is a broadleaf, drought-deciduous species that is shade-intolerant and moderately drought-tolerant. It develops deep taproots to access moisture from deeper soil layers, grows to a height of 30–45 m, and has a wood density of 610–640 kg m^{-3} (Williams, 1990). Western larch is a coniferous deciduous species characterized by its drought tolerance, which is attributed to its extensive root system. As a shade-intolerant

Table 1
30-year climate norms from the period 1981 – 2010 for species and provenances included in the study. MAT: mean annual temperature (°C), MAP: mean annual precipitation (mm), GSP: growing season precipitation (May to September, mm), RH: relative humidity, elevation (m), CMD: Hargreaves climatic moisture deficit (mm). Derived from Climate NA (Wang et al., 2016).

Species	Provenance	MAP	GSP	MAT	RH	Elevation	CMD
Black walnut	Maryland	1228	568	13.8	67	30	118
	Indiana	1059	548	11.0	64	165	131
Western larch	Moist-Cool	712	285	3.2	64	1425	189
	Dry-Hot	568	227	4.6	66	1200	280
	Dry-Cool	929	302	3.5	64	1500	188
Douglas-fir	Coast Range	1782	237	10.8	65	274	361
	Inland	1536	168	11.1	65	205	453
	Cascade foothills	1680	297	10.6	66	486	312

species, it thrives in open environments, making it well-suited for disturbed habitats such as post-fire landscapes. It typically grows to heights of 30–60 m, with an average wood density ranging from 550 to 600 kg m^{-3} (Schmidt et al., 1990). Coastal Douglas-fir is an evergreen conifer characterized by intermediate shade-tolerant seedlings. While drought tolerance varies across populations, it is generally regarded as moderately drought tolerant. It can grow to heights exceeding 75–100 m, with a wood density averaging 450–530 kg m^{-3} (Herman et al., 1990).

Within each species we addressed the following questions using provenances from diverse climatic conditions across the range of each species:

(I) Does second-year biomass allocation reflect memory driven changes in water uptake capacity and transpiration demand associated with drought priming?
(II) Does drought memory increase the number of root tips and specific root length of new roots (SRL)?
(III) Does the formation and effects of drought memory vary among ecophysiologically different species?
(IV) Do changes in second-year growth allocation affect the transpiration and photosynthetic rates of seedlings during the early establishment phase?

We hypothesized that:

(I) Seedlings will increase the biomass allocation to new roots and reduce the allocation to new foliage during the second-year drought, improving the balance between water uptake capacity and transpiration demand due to drought priming during the germination year.
(II) Newly formed roots will have a higher SRL and number of root tips in response to drought priming, improving the efficiency of water and nutrient uptake of the root system.
(III) The magnitude of the effects of drought memory on the new growth allocation and physiology will be higher in species from drier environments (western larch and Douglas-fir). Similarly, ecotypes from drier regions within each species distribution will be more responsive to the second-year drought due to drought memory.
(IV) Seedlings will show higher photosynthesis and transpiration rates during the second-year drought because of greater new root allocation.

To address these hypotheses, we designed a series of three simulated outplanting experiments in the greenhouse that allowed us to compare the formation and effects of drought memory on second-year seedling development under controlled environmental conditions.

2. Materials and methods

2.1. Seedling production

Black walnut, western larch and Douglas-fir seeds were collected from several provenances across their range, spanning a gradient of aridity. Western larch seeds were selected across a biogeoclimatic gradient in British Columbia (Canada; (Meidinger and Pojar, 1991), Douglas-fir provenances range from the coast of Oregon to the windward side of the Cascade mountains, and black walnut seeds were collected from Indiana and Maryland. Specific differences in environmental conditions across provenances are described in Table 1. All species were sown in spring 2020. Black walnut seedlings were grown at Purdue University (West Lafayette, Indiana USA), western larch seedlings were grown at University of Idaho (Moscow, Idaho USA), and Douglas-fir seedlings were grown at Pacific Regeneration Technologies Inc. operational greenhouse in Oregon USA. Douglas-fir seedlings were transported to the University of Idaho on June 10 for inclusion in drought priming treatments alongside western larch. Due to morphological, phenological and physiological differences, the growing conditions and container sizes differed among species. For instance, black walnut seedlings develop prominent taproots, requiring larger containers compared to the other two species. The media, fertilization rate, container size and sowing dates for each species are described in Table 2. Drought priming treatments were applied at the beginning of the rapid growth phase, shortly after germination (7, 6.5 and 14 weeks for black walnut, western larch, and Douglas-fir, respectively) as controlled moisture stress. Seedlings of each species were exposed to three levels of gravimetric moisture contents corresponding to a control, moderate, and high drought stress. The specific gravimetric ranges and corresponding predawn and mid-day leaf water potential (Ψ_{PD} and Ψ_{MD}, respectively) measured at the driest point with a pressure chamber (Model 600 PMS Instrument Corp., Corvallis, Oregon) for each species are described in Table 2. Due to an error during the drought priming application, the Ψ_{PD} and Ψ_{MD} measurements for the moderate treatment in Douglas-fir were taken 4 days too early. As a result, the values reported for this treatment level in Table 2 do not accurately represent the drought stress experienced by the seedlings. Target gravimetric ranges were maintained by weighing containers daily and watering seedlings when containers reached the minimum weight for each drought treatment. New saturated container weights were calculated monthly to adjust for increases in seedling mass. The average temperature and relative humidity for each species is described in Table 2. At the end of the growing season seedlings were removed from the greenhouse and stored at −2.2°C until the simulated outplanting test.

Table 2
First year growing conditions for black walnut, western larch, and Douglas-fir seedlings. RH = relative humidity, Ψ_{MD}: mid-day water potential, Ψ_{PD}: predawn water potential.

Species	Black walnut	Western larch	Douglas-fir
Location	West Lafayette, IN, USA	Moscow, ID, USA	Moscow, ID, USA
Container	D25 (0.47 L)	415 C Styroblock® 0.13 L	615 A Styroblock® 0.34 L
Fertilization	7 g/L Osmocote® Plus 15–9–12 12–14	7 g/L Osmocote® Plus 15–9–12 12–14	35 ppm N (20−7−19) + 45 ppm N (Calcium Nitrate 15.5 % N)
Growing media	Berger© BM8	Berger© BM8	Sungro© Forestry Mix
Sowing date	May 1, 2020	March 18, 2020	February, 2020
Mean temperature	24.8 °C	21.7 °C	21.7 °C
Mean RH	70.5 %	66.5 %	66.5 %
Drought priming application	June 19- Oct 15	June 1- Nov 30	June 11-Nov 30
Priming gravimetric range	Control: 90 %-100 % Moderate: 80 %−95 % High: 65 %−90 %	Control:75 %-100 % Moderate: 60 %−75 % high: 55 %−65 %	High: 75 % - 100 % Moderate: 60 % - 65 % High: 50 % - 55 %
Priming Ψ_{MD} (-MPa)	Control: 0.95 Moderate: 1.27 High: 1.74	Control: 0.62 Moderate: 1.02 High: 1.44	Control: 0.54 Moderate: 0.38 High: 1.27
Priming Ψ_{PD} (-MPa)	Control: 0.32 Moderate: 0.36 High: 0.76	Control: 0.23 Moderate: 0.36 High: 0.66	Control: 0.30 Moderate: 0.30 High: 0.48

2.2. Simulated outplanting

Impacts of drought priming on morpho-physiological development were assessed the following growing season by transplanting seedlings individually into tree containers for the second season of growth and experimental drought exposure. The second growth season occurred in the greenhouse at Purdue University where moisture, temperature, and light were controlled. Nine seedlings per species × provenance × drought priming combination ($n = 216$) were transplanted into 10.65 L tree containers, 40 cm tall and 20 cm in diameter (TPOT4, Stuewe & Sons, Inc., Corvallis, Oregon) to allow for extensive root development. Growing media was a 5:1 mix of high drainage Berger BM2 propagation mix and coarse perlite. To maximize moisture uniformity within and across containers at the time of planting, water was mixed into bulk soil media at a ratio of 160 mL per 1 L of media mix. No additional fertilizer was provided during the simulated outplanting. All seedlings were watered with 100 mL at planting, applied directly to the root plug. Signs of wilting led us to apply an additional 100 mL of water to Douglas-fir and western larch seedlings two weeks after planting to prevent extreme drought stress. No additional water was supplied until the end of the experiment. Containers were wrapped in 0.8 cm thick reflective insulation (R-21, Reflectix, Inc., Markleville, Indiana) to avoid high root zone temperature due to insolation of the tree containers. Seedlings were randomly relocated weekly during the experiment to minimize effects of potential differences in environmental conditions in the greenhouse. Due to phenological differences among species (i.e. budbreak and root development), transplanting occurred at different times during the growing season, aligning with the traditional outplanting periods in their respective native ranges. The transplanting dates were March 8, April 19, and May 29, of 2021, for Douglas-fir, western larch, and black walnut, respectively. The day of budbreak was registered for all seedlings every other day starting at planting. Seedlings were considered to have initiated budbreak when any portion of the enclosed foliage became visible. Finally, all physiological and morphological measurements were taken when roots of each species reached the bottom of the container to avoid changes in root growth patterns due to root interactions with container walls. Root contact with the bottom of the pot was determined by scanning the tree pots containing the plants using an X-ray computed tomography system that allowed us to visualize the root systems of the growing seedlings (Herrero-Huerta et al., 2021). The simulated outplanting growing period since transplant was 50, 49, and 36 days for Douglas-fir, western larch, and black walnut, which – in field settings – encompasses a crucial part of the establishment phase during which seedlings must connect to the hydrologic cycle to minimize transplant shock (Grossnickle, 2012). In this study, we examined the effect of drought priming on newly developed leaves and roots during the second drought exposure, with most measurements taken on these tissues. Physiological measurements were taken at the end of the growing period on April 23 – 26, and June 5 – 8, July 1–4, for Douglas-fir, western larch, and black walnut, respectively. Specifically, we measured whole seedling transpiration (E_{plant}), net photosynthesis (A_{net}), stomatal conductance (g_s), Predawn and mid-day leaf water potential (Ψ_{PD} and Ψ_{MD}, respectively) as described below. All physiological measurements were taken simultaneously at two time points. First, under the second-year drought conditions reached by the end of the experiment. Second, after seedlings were watered to media saturation point with 1.5 L of water to rehydrate the seedlings and ameliorate the effects of the drought (recovery period).

2.3. Whole seedling transpiration and water potential

Whole seedling transpiration was measured on intact seedlings under second-year drought conditions ($E_{plant}D$) and after the recovery period when seedlings had rehydrated ($E_{plant}W$). To measure seedling E_{plant}, the tops of growth containers were sealed with plastic foil wrapping such that moisture in the containers could escape only through foliage transpiration. Thus, the difference in weight of the container between two time points represents the total water loss through transpiration. For each E_{plant} measurement, the weight of the container was measured at predawn, at 9 am, and at noon. The exact time of measurement was registered for each seedling (t1: predawn, t2: morning, t3 noon). Four containers with the same media and similar moisture composition as the ones containing the seedling were weighed at the same time as control blanks for the loss of water through evaporation independent of the seedling. Measurements were taken in the greenhouse under 80 % PPFD of full sunlight and ranging between 24 and 26 ºC, and 40–70 % humidity across species. E_{plant} was calculated as E_{plant} ($g\ m^{-2}\ s^{-1}$) = $\Delta W_{t3\text{-}t2} \cdot \Delta t^{-1}_{t3\text{-}t2} \cdot$ foliage surface area^{-1} (where $\Delta W_{t3\text{-}t2}$ is the difference in weight between measurements at noon and morning after subtracting the weight loss of the control blanks during the same time period, and $\Delta t_{t3\text{-}t2}$ is the time in seconds between measurements at noon and morning). Pre-dawn and mid-day water potential were measured with a pressure chamber (Model 600 PMS Instrument Corp., Corvallis, Oregon) under drought ($\Psi_{PD}D$, $\Psi_{MD}D$, respectively) and after watering ($\Psi_{PD}W$, $\Psi_{MD}W$, respectively). Ψ_{PD} and Ψ_{MD} measurements were collected between 2 and 3am and noon-1pm, respectively. Measurements were taken on leaves for black walnut and branches for Douglas-fir, western larch.

2.4. Gas exchange

A_{net} and g_s were measured under drought ($A_{net}D$, g_sD) conditions and after the recovery period ($A_{net}W$, g_sW). Measurements were taken on the most mature and fully expanded leaves of each seedling with a Li-Cor 6400XT (Licor Inc., Lincoln, NE, USA) from 10 am to 3 pm at PAR= 1500 μmol m^{-2} s^{-1}, CO_2= 400 μmol mol^{-1}, Fan= 500 μmol s^{-1}, and a 6 cm^2 chamber. For Douglas-fir and western larch the foliage inside the chamber was delineated using a marker, cut from the branch, and measured for surface area using Li-Cor 3100 C Leaf Area Meter (Licor Inc., Lincoln, Nebraska, USA) to adjust gas exchange values for measured foliage area.

2.5. Morphological measurements

Immediately after the final physiological measurements were completed, seedlings were extracted from the containers, egressing roots were cleaned free of media, and seedlings were stored in the freezer at −20 ºC for further analysis. Seedlings were later thawed and segmented into old (first-season) and new (second-season) foliage, stem, root plug (old roots) and new roots. After segmenting the root plug from new roots, it was cleaned of media and stored for further analysis. Projected foliage surface area was measured using a LI-3100C Leaf Area Meter. New roots (i.e. those egressing from the root plug) were cut along the surface of the root plug, suspended in water in a transparent tray and arranged to avoid overlapping tissue, and scanned with an Epson Expression 10000XL color scanner (DPI = 800, Seiko Epson Corporation, Nagano-ken, Japan). Total new root length was obtained by analyzing the images using WinRHIZO™ (Régent Instruments Inc., Canada). To quantify root tips in the egressing root scans, we developed an image analysis pipeline in Python, as WinRHIZO could not handle fragmented roots. Scanned root images were skeletonized to reduce them to single-pixel-wide structures, preserving their topology. Manual validation ensured the accuracy of this process. The number of root tips was calculated by identifying all terminal points (root ends) and subtracting the number of distinct root units to account for false tips created by cuts. Foliage, stems, root plug, and new roots were then dried for a minimum of 48 h at 70 ºC, and their dry weight was measured with a precision scale (AB104-S/FACT Analytical Balance, Mettler Toledo, Columbus, OH; resolution ± 0.01 mg). This data was utilized to calculate specific root length (SRL, root length/root dry weight), specific leaf area (SLA, projected foliage area/foliage dry weight), new root allocation ratio (dry weight of egressing roots/seedling dry weight), new foliage allocation ratio (dry weight of new foliage/seedling dry weight), estimation of seedling biomass prior to simulated outplanting (Dry weight sum of: root plug, stem, and first-year foliage in the case of Douglas-fir). Finally, we calculated the ratio of new root tissue to new foliar tissue (R/F ratio).

2.6. Data analysis

Because drought priming treatments reduced the overall size of seedlings in all species (Fig. S1, Table S1, S2, and S3) the resulting morpho-physiological responses during the second year of drought were assessed primarily through variables that were normalized (i.e. biomass allocation, normalized by seedling total weight or E_{plant} normalized by foliage area). Results that are not essential for the analysis of drought memory are presented in the supplementary material (Fig. S1, Table S1, S2, and S3).

Data was analyzed using species-specific linear mixed-effect models (nlme package in the R statistical environment, version 4.1.2), where the drought priming treatment, provenance, and their interaction were set as independent variables, repetition plant id (1−9) was set as the random effect and measured plant traits as the dependent variable. Normality of model residuals and homogeneity of variance was assessed using quantile-quantile and residuals vs fitted values plots. Datum on some of the plant traits were transformed with a log function (western larch: $A_{net}W$, G_sW; Douglas-fir: R/F, root plug mass, SLA, $E_{plant}W$, $E_{plant}D$, $A_{net}W$; G_sW, G_sD black walnut: R/F, leaves dry mass, and area) or boxcox (Douglas-fir: Budbreak) were log transformed to meet assumptions of normality and homoscedasticity for the linear mixed-effect model. Estimated marginal means and standard error of the mean were obtained from the linear mixed-effect model using the emmeans package (Lenth, 2022; Russell et al., 2022) that also allowed the back-transformation of means and standard errors if data was transformed. We performed Pearson correlations to explore the relationship between new egressing root biomass allocation and physiological processes such as E_{plant} and photosynthesis rate. *Post-hoc* contrasts described in the results section were based on Tukey HSD tests performed through pairwise comparisons (emmeans package).

3. Results

For Douglas-fir and western larch, but not for black walnut, drought priming during the germination year resulted in the formation of a drought stress memory as defined by seedling biomass allocation and physiological traits responding differentially to second-year drought depending on previous drought exposure. Species and provenances differed in their expression of drought stress memory during the second-year drought. Specifically, western larch seedlings exposed to drought priming expressed greater biomass allocation to new roots, whereas

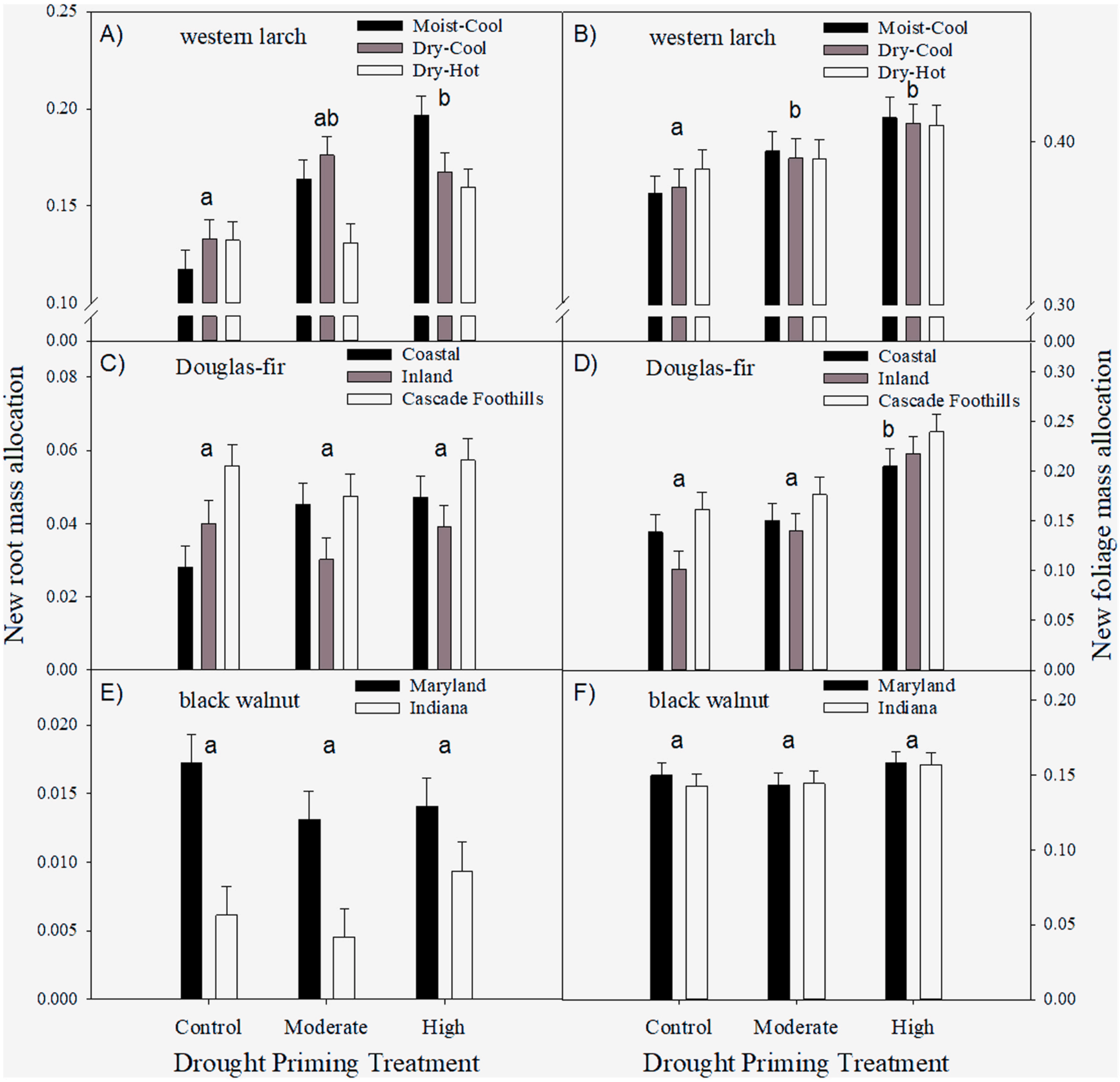

Fig. 1. Post-planting new root (left) and foliage (right) mass allocation response to drought priming treatment (Control, Moderate, and High) across species and provenances. Bars are estimated marginal means and error bars are standard errors of the mean. Different letters above the bar groups indicate significant differences across priming treatments based on Tukey's pairwise comparisons.

Douglas-fir seedlings showed earlier bud break and higher biomass allocation to new foliage, while in black walnut we did not detect signs of drought memory formation.

3.1. Changes in biomass allocation driven by drought memory

3.1.1. Western larch

Moderate to high drought priming resulted in greater allocation of biomass towards new egressing roots in western larch seedlings from the moist-cool and dry-cool provenances (5–8 % and 3–5 % of total seedling biomass, respectively), but not for the hot-dry (Fig. 1A, interaction provenance×treatment $P = 0.004$). High drought priming also increased the number of root tips compared to controls, but this effect was observed exclusively in the moist-cool provenance (Table 3, interaction provenance×treatment $P = 0.006$). Biomass allocation towards new foliage increased across all provenances resulting from high drought priming compared to the control, while no differences were shown for the moderate drought priming ($P < 0.001$; 0.41 ± 0.01, 0.37 ± 0.01, 0.39 ± 0.00, respectively). The ratio of new egressing roots to new foliage dry mass increased in response to high and moderate drought priming in all western larch provenances ($P = 0.001$). The total length and dry weight of the newly produced egressing roots increased in response to drought priming only for the moist-cool provenance, while it was similar across priming treatments for the other two provenances (interactions: provenance×treatment, Table S1). The SRL of the egressing roots was also significantly higher in the seedlings exposed to the high drought priming compared to the controls (24.9 ± 1.03 m/g and 21.1 ± 1.03 m/g, respectively; $P = 0.014$), while moderate drought priming showed no statistical differences (22.5 ± 1.03; Table 3).

Overall, the effect of drought priming treatments varied across provenances (interaction provenance×treatment $P = 0.031$). Newly produced foliage dry mass was reduced by high drought priming in the dry-cool provenance by 25 %, high and moderate priming (statistically equal) for the dry-hot provenance by 21 %, while no significant differences in response to priming were shown for the moist-cool provenance (Table S1). The surface area of new foliage was reduced by high drought priming in the dry-cool provenance by 22 %, but priming treatments had no effects in the remaining provenances (Table S1). The SLA of newly produced foliage did not differ among priming treatments and provenances for western larch seedlings (Table 3).

3.1.2. Douglas-fir

Douglas-fir seedling budbreak and biomass allocation to new foliage varied in response to drought priming treatments, but not for new egressing root allocation, number of root tips, root dry mass, or root length (Fig. 1A, Table 4). Seedlings grown under the high drought priming treatment had an earlier budbreak (Fig. 2, Table S2) and significantly higher biomass allocation to new foliage (0.22 ± 0.01) and SLA (42.6 ± 4.2 cm^2 g^{-1}) compared to moderate (0.16 ± 0.01, 36.4 ± 3.59 cm^2 g^{-1}) and control (0.13 ± 0.01; 29.9 ± 3.0 cm^2 g^{-1}), which showed no difference (Fig. 1, Table 4). The ratio of new egressing roots to new foliage dry was lower for the seedlings exposed to high levels of drought priming, but only in the Inland provenance, and trending in a similar direction in Coastal (Treatment x Provenance interaction, $P = 0.040$; Table 4).

Irrespective of drought priming, biomass allocation to new egressing roots and SRL was significantly higher in seedlings from the Cascade foothills compared to the Coastal provenance, while no differences were found with seedlings from the Inland region (Table 4). The biomass allocation to new foliage was higher on the Cascade foothills provenance than Inland, while the Coastal seedlings did not differ from the other provenances ($P = 0.018$). Overall, the dry mass of new foliage was lower in seedlings from Cascade foothills compared to the other provenances ($P = 0.008$). New foliage area increased in response to the high drought treatment but only in Inland and Coastal provenances (provenance x treatment interactions; $P = 0.021$; Table S2). New foliage SLA was lower in the Cascade foothills seedlings compared to the other provenances that did not differ between each other ($P = 0.040$).

Table 3

Upper side – Western larch seedling characteristics in the second year of growth across provenances and drought priming treatments. SRL: specific root length, SLA: specific leaf area, R/F: new root to new foliage mass ratio, E_{Plant}: whole seedling transpiration rate, A_{net}: net photosynthesis, Ψ_{MD}: mid-day water potential, Ψ_{PD}: predawn water potential. D or W next to a variable means that measurements were taken under drought or after the recovery period, respectively. Data are estimated marginal means and standard errors of the mean. Lower side - Linear mixed-effect models results across drought priming treatments, provenances and their interaction for seedling morphological and physiological variables.

Western larch	Provenance								
	Moist-cool			Dry-cool			Dry-hot		
Priming	Control	Moderate	High	Control	Moderate	High	Control	Moderate	High
SRL	20.5 (1.7)	21.9 (1.7)	26.5 (1.7)	23.9 (1.7)	23.6 (1.7)	24.1 (1.7)	18.9 (1.7)	22.0 (1.7)	24.2 (1.7)
SLA	81.0 (19)	90.6 (19)	88.3 (19)	82.1 (19)	81.6 (19)	85.8 (19)	81.8 (19)	82.9 (19)	84.2 (19)
R/F	0.32 (0.03)	0.42 (0.03)	0.48 (0.03)	0.36 (0.03)	0.45 (0.03)	0.41 (0.03)	0.35 (0.03)	0.35 (0.03)	0.39 (0.03)
Nr root tips	663 (134)	1100 (134)	1268 (134)	1021 (134)	1275 (134)	944 (134)	820 (134)	659 (134)	852 (134)
Ψ_{MD} D	1.29 (0.07)	1.22 (0.07)	1.08 (0.07)	1.33 (0.07)	1.07 (0.07)	1.09 (0.07)	1.21 (0.07)	1.39 (0.07)	1.18 (0.07)
Ψ_{MD} W	0.73 (0.04)	0.8 (0.04)	0.82 (0.04)	0.94 (0.04)	0.88 (0.04)	0.98 (0.04)	0.88 (0.04)	1.02 (0.04)	0.86 (0.04)
E_{Plant} 10^{-5} W	3.9 (0.47)	4.4 (0.47)	5.4 (0.47)	4.6 (0.47)	5.4 (0.47)	5.7 (0.47)	4.7 (0.47)	4.7 (0.47)	4.9 (0.47)
A_{net} W	6.8 (0.6)	7.1 (0.7)	7.4 (0.7)	8.0 (0.8)	8.3 (0.8)	8.6 (0.8)	7.6 (0.8)	7.7 (0.8)	8.0 (0.8)
	Treatment			Provenance			Provenance x Treatment		
Alloc roots	**38.6 (<0.001)**			**7.6 (0.022)**			**15.49 (0.004)**		
Alloc foliage	**15.5 (<0.001)**			0.1 (0.936)			1.0 (0.903)		
SRL	**8.5 (0.014)**			2.8 (0.247)			4.3 (0.365)		
SLA	2.6 (0.279)			2.0 (0.366)			2.4 (0.658)		
R/F	**13.3 (0.001)**			4.7 (0.094)			8.8 (0.073)		
Nr root tips	3.4 (0.186)			**9.6 (0.008)**			**14.6 (0.006)**		
Ψ_{MD} D	**8.1 (0.017)**			3.2 (0.204)			**11.4 (0.023)**		
Ψ_{MD} W	1.9 (0.379)			**21.7 (<0.001)**			**10.3 (0.035)**		
E_{Plant} 10^{-5} D	**9.6 (0.008)**			4.1 (0.128)			3.0 (0.561)		
E_{Plant} 10^{-5} W	**6.7 (0.036)**			3.9 (0.144)			2.8 (0.590)		
A_{net} D	0.5 (0.769)			5.2 (0.074)			4.7 (0.317)		
A_{net} W	0.8 (0.664)			4.2 (0.122)			0.0 (0.999)		

Units: SRL (m g^{-1}), SLA (cm^2 g^{-1}), R/F (g g^{-1}), Ψ (-MPa), E_{Plant} (Kg m^{-2} s^{-1}), A_{net} (μmol CO_2 m^{-2} s^{-1})

Table 4

Upper side - Douglas-fir seedling characteristics in the second year of growth across provenances and drought priming treatments. SRL: specific root length, SLA: specific leaf area, R/F mass ratio: new root to new foliage ratio, E_{Plant}: whole seedling transpiration rate, A_{net}: net photosynthesis, Ψ_{MD}: mid-day water potential, Ψ_{PD}: pre-dawn water potential. D or W next to a variable means that measurements were taken under drought or after the recovery period, respectively. Data are estimated marginal means and standard errors of the mean. Lower side - Linear mixed-effect models results across drought priming treatments, provenances, and their interaction for seedling morphological and physiological variables.

Douglas-fir	Provenance								
	Coastal			Inland			Cascade foothills		
Priming	Control	Moderate	High	Control	Moderate	High	Control	Moderate	High
SRL	16.2 (1.3)	15.4 (1.3)	16.0 (1.3)	16.9 (1.3)	13.3 (1.3)	14.8 (1.3)	19.3 (1.3)	17.5 (1.3)	19.7 (1.3)
SLA	30.5 (5.2)	39.8 (6.8)	52.5 (9.0)	31.7 (5.7)	40.3 (6.9)	48.7 (8.3)	27.7 (4.7)	30.1 (5.1)	30.1 (5.1)
R/F	0.20 (0.05)	0.39 (0.06)	0.25 (0.06)	0.40 (0.07)	0.25 (0.06)	0.18 (0.05)	0.38 (0.06)	0.30 (0.06)	0.24 (0.06)
Nr root tips	374 (76.6)	475 (76.6)	414 (76.6)	500 (76.6)	289 (76.6)	322 (76.6)	508 (76.6)	419 (76.6)	392 (76.6)
Ψ_{MD} D	1.73 (0.07)	1.60 (0.07)	1.44 (0.07)	1.62 (0.07)	1.52 (0.07)	1.45 (0.07)	1.56 (0.07)	1.68 (0.07)	1.46 (0.07)
Ψ_{MD} W	0.85 (0.04)	0.83 (0.04)	0.83 (0.04)	0.89 (0.04)	0.81 (0.04)	0.69 (0.04)	0.79 (0.04)	0.86 (0.04)	0.74 (0.04)
E_{Plant} 10^{-5} W	4.0 (0.4)	4.4 (0.4)	3.8 (0.4)	5.0 (0.6)	4.0 (0.4)	3.5 (0.4)	5.9 (0.6)	5.3 (0.6)	5.6 (0.6)
A_{net} W	3.5 (0.5)	3.6 (0.5)	4.5 (0.7)	3.1 (0.5)	3.3 (0.5)	4.2 (0.6)	5.0 (0.8)	5.4 (0.9)	6.8 (1.0)
	Treatment			Provenance			Provenance x Treatment		
Alloc roots	2.6 (0.268)			**13.9 (0.001)**			6.8 (0.146)		
Alloc foliage	**40.8 (<0.001)**			**8.0 (0.018)**			2.2 (0.681)		
SRL	3.5 (0.170)			**13.3 (0.001)**			1.6 (0.814)		
SLA	**6.2 (0.045)**			**6.4 (0.040)**			2.0 (0.7378)		
R/F	5.0 (0.081)			0.5 (0.775)			**10.0 (0.040)**		
Nr root tips	2.0 (0.360)			1.3 (0.517)			4.5 (0.344)		
Ψ_{MD} D	**12.0 (0.003)**			1.2 (0.562)			4.7 (0.324)		
Ψ_{MD} W	**9.0 (0.011)**			2.1 (0.349)			8.0 (0.091)		
E_{Plant} 10^{-5} D	3.5 (0.173)			**34.0 (<0.001)**			5.2 (0.267)		
E_{Plant} 10^{-5} W	2.9 (0.239)			**18.0 (<0.001)**			3.2 (0.526)		
A_{net} D	**16.8 (<0.001)**			**44.4 (<0.001)**			4.3 (0.366)		
A_{net} W	**6.1 (0.048)**			**16.5 (<0.001)**			0.0 (0.999)		

Units: SRL (m g^{-1}), SLA (cm^2 g^{-1}), Ψ (-MPa), E_{Plant} (Kg m^{-2} s^{-1}), A_{net} (μmol CO_2 m^{-2} s^{-1})

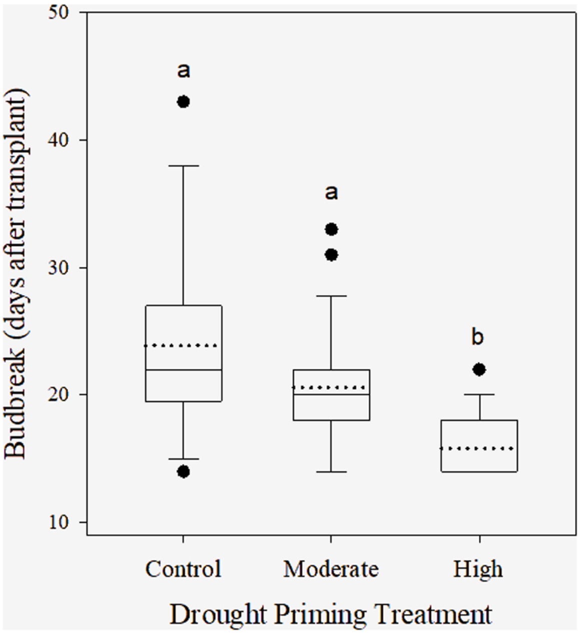

Fig. 2. Budbreak date in one year old Douglas-fir seedlings measured as the number of days after transplant. The horizontal continuous and dotted lines in the boxplots represent the mean and median budbreak values, respectively. Different letters above the bar groups indicate significant differences across priming treatments based on Tukey's pairwise comparisons.

3.1.3. *Black walnut*

Black walnut seedlings biomass allocation to new roots, number of root tips, and foliage were unaffected by drought priming treatments (Fig. 1, Table 5). New root allocation was significantly higher in seedlings from Maryland compared to Indiana ($P < 0.001$; 0.015 ± 0.001 and 0.007 ± 0.001, respectively), but whole foliage allocation did not differ between provenances (Fig. 1E, 3D). The ratio of new roots to new foliage dry mass, biomass allocation of new egressing roots, and the overall dry mass and length of new roots were higher in the seedlings from Maryland compared to Indiana (Table 5). Neither biomass allocation, SLA, nor SRL differed among provenances or priming treatments (Table 5). Foliar biomass and foliage surface area was greater in control compared to primed seedlings ($P = 0.047$; Table S3). Seedlings from Maryland had higher new root mass ($P < 0.001$) and root length ($P < 0.001$) than Indiana (0.62 ± 0.05 g vs 0.29 ± 0.05 g and 15.3 ± 2.0 m vs 6.0 ± 0.8 m, respectively; Table S3).

3.2. *Seedling transpiration, photosynthesis, and water potential*

3.2.1. *Western larch*

Western larch seedlings exposed to high drought priming showed greater E_{plant} than controls under second year drought and after the recovery period when seedlings had rehydrated (Fig. 3B). Seedlings showed a less negative Ψ_{MD} at the end of the second-year drought, except for seedlings from the dry cool provenance. Differences in Ψ_{MD} were not apparent after the recovery period (Table 3). Ψ_{PD} did not differ across treatments under the second-year drought or after the recovery period (Table S1). No differences in A_{net} were detected among priming treatments (Fig. 3A), and no differences were detected across provenances in E_{plant} or A_{net} or g_s, except for the dry-cool provenance, which showed higher g_s than the dry-hot provenance under drought conditions (Figs. 3A, 3B, Table 3, S1).

3.2.2. *Douglas-fir*

Douglas-fir seedlings exposed to high drought priming had significantly greater A_{net} (Fig. 3B) and less negative Ψ_{MD} and Ψ_{PD} under the second-year drought and after the recovery period (Table 4). g_s followed the same pattern under second-year drought conditions, while differences were not apparent after the recovery period. Seedlings from Cascade had higher E_{plant} and A_{net}, and g_s than the other provenances under second-season drought and after the recovery period, while Coastal and Inland seedlings did not differ significantly (Figs. 3C, 3D,

Table 5

Upper side - Black walnut seedling characteristics in the second year of growth across provenances and drought priming treatments. SRL: specific root length, SLA: specific leaf area, R/F mass ratio: new root to new foliage ratio, E_{Plant}: whole seedling transpiration rate, A_{net}: net photosynthesis, Ψ_{MD}: mid-day water potential, Ψ_{PD}: pre-dawn water potential. D or W next to a variable means that measurements were taken under drought or after the recovery period, respectively. Data are estimated marginal means and standard errors of the mean. Lower side - Linear mixed-effect models results across drought priming treatments, provenances, and their interaction for seedling morphological and physiological variables.

Black Walnut	Provenance					
	Maryland			Indiana		
Priming	Control	Moderate	High	Control	Moderate	High
SRL	30.6 (2.8)	30.1 (2.8)	28.9 (2.8)	28.6 (2.8)	26.4 (2.8)	29.3 (2.8)
SLA	139 (6.07)	148 (6.07)	140 (6.07)	145 (6.07)	140 (6.07)	149 (6.07)
R/F	0.10 (0.025)	0.07 (0.017)	0.079 (0.019)	0.03 (0.007)	0.023 (0.007)	0.05 (0.011)
Nr root tips	1139 (137)	870 (137)	842 (137)	413 (137)	281 (137)	609 (137)
Ψ_{MD} D	1.42 (0.06)	1.32 (0.06)	1.44 (0.06)	1.36 (0.06)	1.48 (0.06)	1.45 (0.06)
Ψ_{MD} W	1.02 (0.06)	1.1 (0.06)	1.12 (0.06)	1.26 (0.06)	1.32 (0.06)	1.13 (0.06)
E_{Plant} 10^{-5} W	2.2 (0.15)	1.9 (0.15)	1.9 (0.15)	1.5 (0.15)	1.6 (0.15)	1.8 (0.15)
A_{net} W	8.5 (0.6)	6.7 (0.6)	7.3 (0.6)	6.3 (0.6)	5.6 (0.6)	6.4 (0.6)

	Treatment	Provenance	Provenance x Treatment
Alloc roots	2.6 (0.278)	**22.9 (<0.001)**	2.4 (0.297)
Alloc foliage	3.3 (0.194)	0.1 (0.713)	0.3 (0.854)
SRL	0.2 (0.891)	0.6 (0.445)	0.5 (0.771)
SLA	0.2 (0.920)	0.2 (0.626)	2.2 (0.326)
R/F	1.9 (0.381)	**19.1 (<0.001)**	2.4 (0.309)
Nr root tips	4.6 (0.097)	**24.0 (<0.001)**	3.1 (0.209)
Ψ_{MD} D	1.1 (0.589)	0.5 (0.467)	4.1 (0.131)
Ψ_{MD} W	2.2 (0.339)	**9.8 (0.002)**	4.0 (0.138)
E_{Plant} 10^{-5} D	0.3 (0.881)	**14.0 (<0.001)**	**6.2 (0.044)**
E_{Plant} 10^{-5} W	1.4 (0.492)	**10.9 (<0.001)**	**5.2 (0.073)**
A_{net} D	2.5 (0.281)	**10.44 (0.001)**	1.8 (0.413)
A_{net} W	**6.2 (0.044)**	**11.8 (<0.001)**	2.2 (0.329)

Units: SRL ($m\ g^{-1}$), SLA ($cm^2\ g^{-1}$), Ψ (-MPa), E_{Plant} ($g\ s^{-1}\ m^{-2}$), A_{net} (μmol $CO_2\ m^{-2}\ s^{-1}$)

Table 4 and S2).

3.2.3. *Black walnut*

E_{plant}, Ψ_{MD} and Ψ_{PD} of black walnut seedlings were unaffected by drought priming under all second-year conditions. A_{net} and g_s followed the same pattern, although control seedlings showed a slightly higher A_{net} and g_s than moderate drought conditioning after the recovery period (Fig. 3E, Table 5). Seedlings from Maryland showed a higher E_{plant} and A_{net} compared to seedlings from Indiana under all conditions (Fig. 3E, 3F, Table 5).

3.3. *Biomass allocation and seedling gas exchange*

Biomass allocation to new roots was positively correlated to E_{plant} and A_{net} under drought and after the recovery period in all species when analyzed independently of provenance and drought priming (Fig. 4). Western larch was the only exception to this result since the correlation between new root biomass allocation and A_{net} was not significant ($P = 0.35$ and $P = 0.75$). The correlation strength varied across species, and between drought and well-watered conditions. Overall, black walnut (Figs. 4E, 4F) showed the strongest correlation between new root allocation and E_{plant} and A_{net}, followed by Douglas-fir (Figs. 4C, 4D) and western larch (Figs. 4A, 4B). This correlation was also stronger under drought than after the recovery period when seedlings had rehydrated (Fig. 4).

4. Discussion

Our results demonstrate that Douglas-fir and western larch seedlings developed drought memory in response to drought exposure during the first year of growth, while black walnut did not. Seedling development under the second-year drought depended on drought priming in the two conifer species, including complex interactions between morphological and physiological traits. We found that the drought priming gradient yielded species-specific morpho-physiological responses upon exposure to drought in the subsequent growing season. Overall, western larch showed an increase in biomass allocation towards new egressing roots with greater drought priming intensity during germination year, which allowed for higher water uptake relative to seedling demand as indicated by a higher E_{plant}. Douglas-fir seedlings showed earlier bud break in response to drought priming accompanied by an increase in A_{net} and biomass allocation to new foliage, while no effects were detected in biomass allocation to roots. Finally, black walnut did not show any changes in biomass allocation or physiology in response to priming. These results highlight the crucial role that drought memory plays in the adaptive responses of trees to recurrent droughts across different forest ecosystems.

4.1. *Changes in biomass allocation and gas exchange in response to drought memory*

Our first hypothesis was partially rejected because the only species that showed an improvement in the balance between water uptake capacity and transpiration demand in response to drought priming was western larch, as illustrated by the increased ratio of new roots to new foliage biomass (Table 3, Fig. 1A-B). The memory-driven acclimation to drought in western larch was mainly expressed by a greater allocation to new roots (Fig. 1A). Increased allocation to new roots enhances root-soil contact that connects newly planted seedlings to the hydrologic cycle before severe drought occurs. Evidence of morphological acclimation driven by drought memory across successive growing seasons was recently reported in other tree species. *Pinus ponderosa* seedlings developed greater root biomass during a second-year simulated drought if they were drought primed during germination year (Pinto et al., 2023). Drought primed *Fagus sylvatica* seedlings developed greater root biomass even in the absence of second-year drought (Yang et al., 2022), showing that drought memory can result in a sustained development of a stress resistant phenotype for a certain time after the drought stress has stopped (Lämke and Bäurle, 2017; Walter et al., 2013). Although these studies did not report data on new growth allocation driven by drought

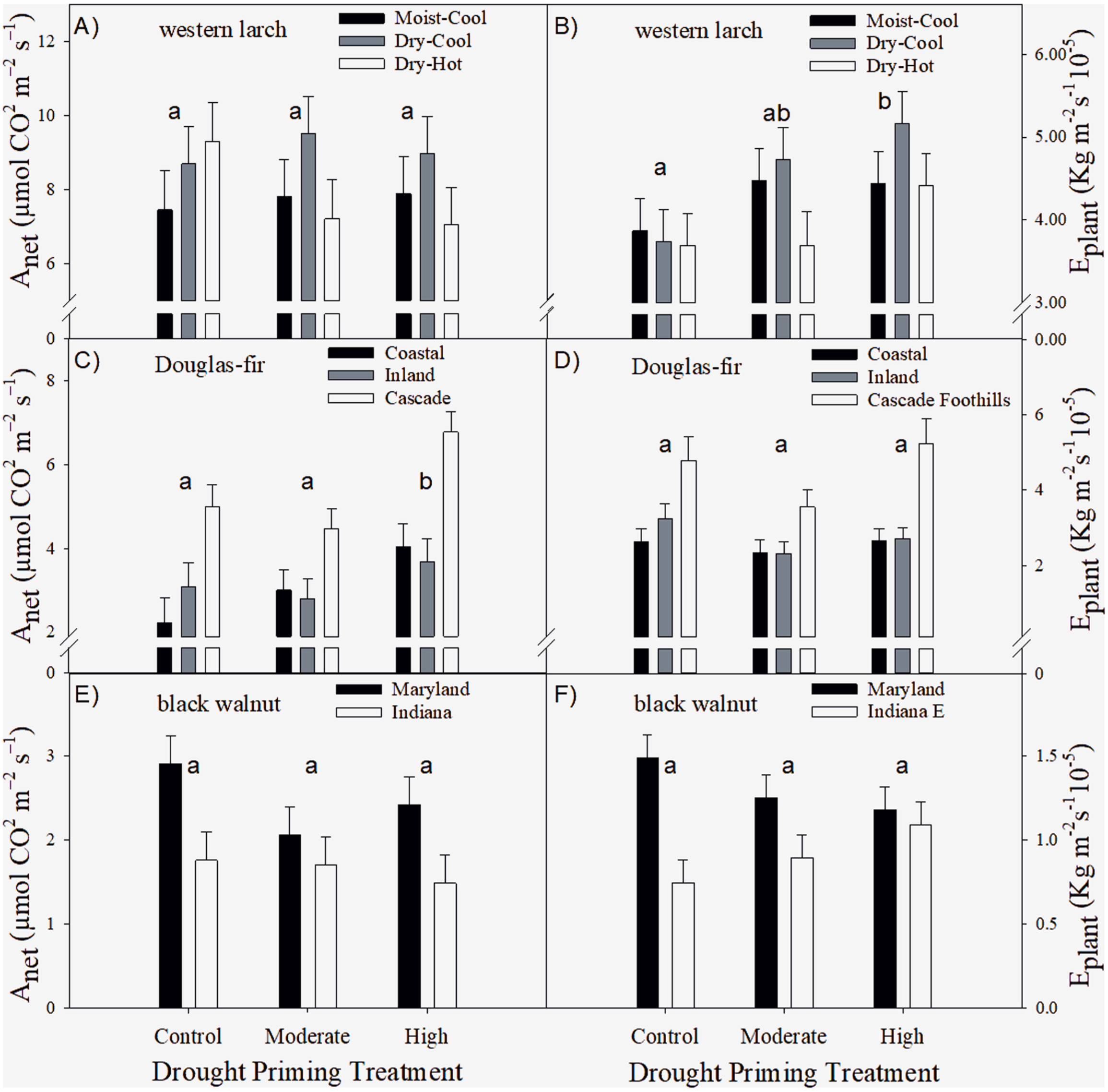

Fig. 3. Post-planting A_{net} (net photosynthesis, left) and E_{Plant} (whole seedling transpiration rate, right) responses to drought priming treatment (Control, Moderate, and High), measured under a subsequent second-year drought across species and provenances. Bars are estimated marginal means and error bars are standard errors of the mean. Different letters above the bar groups indicate significant differences across priming treatments based on Tukey's pairwise comparisons.

memory, it suggests that a similar mechanism of drought acclimation is shared across phylogenetically different species. This result adds a new dimension to the functional equilibrium hypothesis (Brouwer, 1983), where biomass allocation to roots and shoots is not only controlled by current growing conditions but also by drought priming across growing seasons.

In addition to the increase in new root allocation, and as expected in our second hypothesis, the newly formed roots in western larch seedlings had higher SRL, and, in one of the provenances, higher number of root tips (Table 3). New egressing roots of greater SRL and continued production of new root tips explore larger soil volumes and, thus, improve the efficiency of water and nutrient uptake per unit of root dry weight (Comas et al., 2013; Reich et al., 1998; York et al., 2013). Seedlings with higher SRL have been shown to have a higher root proliferation rate (Eissenstat, 1991), which can further increase their foraging capacity and the chance for successful establishment. In agreement with our second hypothesis, the increase in biomass allocation and SRL in high drought primed western larch seedlings was concurrent with less negative Ψ_{MD}D during the second-year drought while supporting a higher E_{plant}D (Table 3). Furthermore, Ψ_{PD}D was similar across drought priming treatments suggesting that western larch drought memory improves water uptake capacity as well as osmotic adjustment. Although A_{net} was similar across priming treatments (Fig. 3A, Table 3), the increase in E_{plant} (Fig. 3B) induced by drought memory suggests that, in the absence of other limitations to $A_{net,}$ these seedlings had overall higher carbon assimilation. Similar to our results, drought priming did not affect the A_{net} of *Picea glauca* under a subsequent drought, and it impaired it in *Acer saccharum* (Ribeyre et al., 2022). However, that study evaluated these correlations within the same growing seasons, which increases the risk of cumulative drought damage. Thus, as we expected in our third hypothesis, upon exposure to a second round of drought, the earlier formation of a drought memory triggered morpho-physiological mechanisms in western larch seedlings that increased their water uptake capacity to meet transpiration demand.

The development of new foliage is also crucial in balancing seedling water demand relative to uptake capacity. Contrary to our expectations in hypothesis 1, Douglas-fir increased new foliage biomass allocation in response to drought priming, while maintaining a similar new biomass allocation to roots across treatments (Table 4, Figs. 1C, 1D).

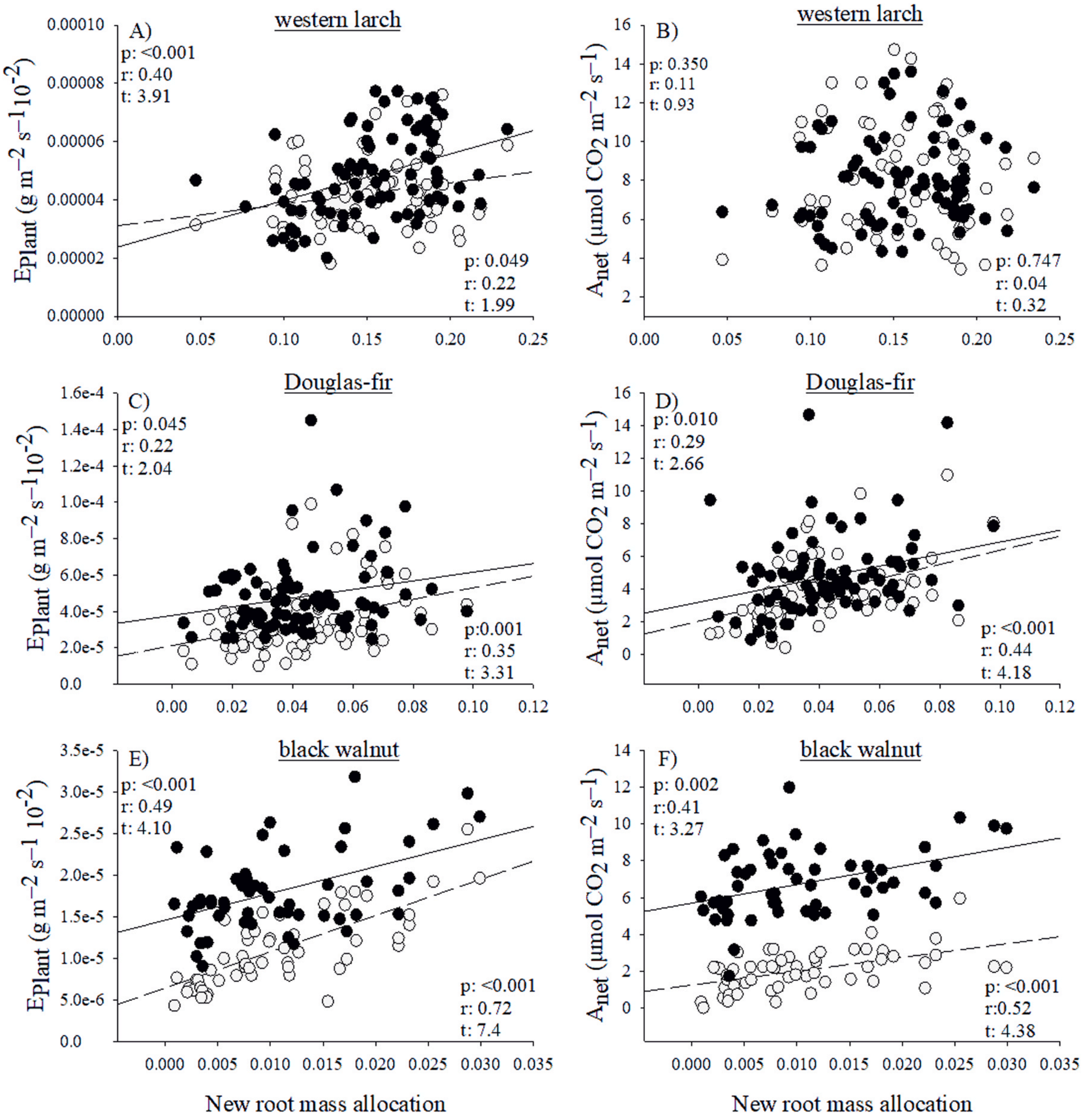

Fig. 4. Relationships between new root mass allocation (dry weight of egressing roots/seedling dry weight) and whole seedling transpiration rate (E_{plant}; leftside) and photosynthesis rate (A_{net}; right side) for one year old western larch (A, B), Douglas-fir (C, D), and black walnut (E, F) analyzed independently of provenance and drought priming treatments. Measurements were taken under second-year growing season drought (○) and after the recovery period when seedlings had rehydrated (●). Statistical analysis values on the lower right side of each graph and dashed regression line correspond to second year drought conditions and upper left values and solid regression line for the same correlation after watering the seedlings to optimal levels.

Furthermore, the ratio between new root to new foliage biomass decreased in two out of three provenances of Douglas-fir (Table 4). This contrasts with species such as *Abies alba* (Yang et al., 2022) and *Argania spinosa* (de la Fuente et al., 2023), which did not show any change in allocation to new roots or foliage in response to drought priming. The increase in foliage allocation could be explained in part by the earlier budbreak, which allowed for more time for these seedlings to develop new needles (Fig. 2). Earlier budbreak could also explain the higher A_{net} observed in Douglas-fir seedlings that were exposed to high drought priming compared to controls (Fig. 3C). Differences in A_{net} across priming treatments decreased once seedlings were watered, suggesting that previous exposure to drought can increase the photosynthetic rate during a subsequent drought. The maintenance of a higher A_{net} during recurrent drought stress has been attributed in several species to mechanisms such as memory driven accumulation of Ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and chlorophyll (Mukiri et al., 2023). Given that the main source of carbon for new root development in Douglas-fir is current photosynthate (van den Driessche, 1987), the rapid development of foliage early in the growing season, when water is readily available, could be a successful strategy to support root growth before the dry season (Villar-Salvador et al., 2012). In addition, an increase in non-structural carbohydrates improves the osmotic adjustment capacity of plants (McDowell, 2011; Sala et al., 2012), which could explain why Douglas-fir seedlings exposed to drought priming maintained a less negative Ψ_{MD} under second-year drought conditions and after watering (Table 4). This strategy might be employed by other species like *Quercus robur*, which showed an earlier shoot flush in response to previous exposure to drought, as well as a higher concentration of osmotically active carbohydrates (Spieß et al., 2012).

In contrast with the previous two species, black walnut did not show any changes in root or shoot biomass allocation or gas exchange in response to drought priming (Figs. 1E, 1F, Table 5). As presented previously, within the few studies assessing the effect of drought memory on morpho-physiological acclimation, there are reports of species that showed no changes in the allocation of biomass towards roots and shoots (de la Fuente et al., 2023; Villar-Salvador et al., 2013; Yang et al., 2022) or in gas exchange (Ribeyre et al., 2022).

The results described above indicate that species like Douglas-fir and Western larch can form drought memory and modify their morpho-physiological development following a drought priming event. However, our experimental design does not allow us to distinguish whether the observed responses during the second growing season result from a sustained memory-driven response (Type 1 stress memory), are triggered by the second-year drought (Type 2 stress memory), or represent a combination of these strategies depending on the specific trait measured. In addition, the lack of response to drought priming in black walnut seedlings does not mean that the species does not form and express memory in traits that we have not considered in this experiment. Drought severity during priming is a critical factor in the formation and expression of drought memory and is likely species-specific (Vilagrosa et al., 2003). In our experiment, the moderate priming treatment was generally too mild (Table 2), leading to less frequent formation and expression of memory-driven drought acclimation. Additionally, because simulated outplanting experiments performed under controlled environmental conditions are expected to reveal more pronounced memory-driven responses (Puértolas et al., 2024), further research is needed to assess the impact of memory-driven acclimation under field conditions, where environmental variability is greater.

4.2. *Species ecology could drive the effects of drought memory*

Due to phenological differences during the second growing season and some variations in nursery growing conditions across species (as detailed in Table 2), our study did not allow for a statistical comparison across species. However, because of the overall similar experimental conditions (Table 2), we can qualitatively identify and compare patterns in species response to drought priming. In agreement with our third hypothesis, Douglas-fir and western larch, the two species inhabiting drier and more heterogeneous environment (Table 2), showed a higher memory driven adaptative phenotypic plasticity in response to drought. The lack of morpho-physiological responses driven by drought memory described in black walnut and other species like *Abies alba* (Yang et al., 2022) or *Argania spinosa* (de la Fuente et al., 2023), could be related to the inherent cost of phenotypic plasticity across different environments (Murren et al., 2015). High phenotypic plasticity offers considerable adaptive advantages in heterogeneous environments. In contrast, memory driven plasticity has strong potential to yield maladaptive trait values in species like black walnut from environmentally mild and homogeneous regions (Table 2) where suboptimal phenotypes could arise following drought, thus reducing fitness under favorable conditions due to lower growth and competitive capacity (Puértolas et al., 2024). To prevent the development of suboptimal phenotypes in homogeneous environments, memory dissipation or forgetfulness is likely to play an important role in maximizing growth under favorable conditions as soon as an infrequent stress event ends (Crisp et al., 2016). In contrast, species like Douglas fir and western larch, which frequently face drought during the growing season (GSP, Table 1), have developed memory-driven mechanisms to cope with low water availability and its effects (McDowell et al., 2008), providing the chance for trees to acclimate to their local conditions.

Seed provenance significantly influenced morpho-physiological traits (Table 3–S3), with its impact on the formation and expression of drought memory varying across species, as shown by provenance by treatment interactions. Douglas-fir showed high responsiveness to drought priming and significant variation across provenances, though interactions were limited (e.g., new foliage area increased with drought priming in all provenances except Cascade Foothills, Table S3). Western larch from hot-dry regions showed a trend toward increased root biomass allocation under drought priming, but this was not significant compared to other provenances with significant increases (Fig. 1A, Table 3). Similar provenance-drought priming interactions were reported in *Eucalyptus globulus* (Guarnaschelli et al., 2003). In our study, black walnut provenance explained almost all variation in traits and had no impact on drought memory. These findings suggest that the relative adaptive value of memory-driven plasticity discussed earlier across species also applies to provenances within species that have large and variable distribution ranges.

One additional source of variation in memory driven acclimation across species is phylogeny. Gymnosperms have key life history differences compared to Angiosperms as well as different genetic and epigenetic mechanisms, larger genomes and larger amounts of non-coding DNA (Fossdal et al., 2024). These characteristics may contribute to gymnosperms relying on epigenetic memory to enhance their phenotypic plasticity and ability to acclimate to recurrent stress (Yakovlev et al., 2012; Fossdal et al., 2024). Although our experiments involve a limited number of species, the observed response patterns align with this trend. Studies involving a larger and more diverse set of species are necessary to confirm these findings and better understand the underlying mechanisms of stress memory across angiosperms and gymnosperms.

4.3. *Biomass allocation and seedling gas exchange*

In accordance with the fourth hypothesis, one of our main results is that biomass allocation is a key component in determining the water uptake capacity and gas exchange of seedlings during the early stages of establishment. For all three species, new root allocation was positively correlated to E_{plant} under drought conditions and after the recovery period when seedlings had rehydrated (Figs. 4A, 4C, 4E). Similarly, an increase in new root allocation was associated with higher A_{net} in black walnut and Douglas-fir (Figs. 4B, 4D, 4F). Although the relationship between new root allocation and A_{net} was not significant in western larch, seedlings with higher new root allocation had higher E_{plant}, which translates into a higher amount of carbon assimilation over the three-hour period that E_{plant} was measured in the absence of other limitations to A_{net}. E_{plant} and A_{net} are complex processes that depend, among other factors, on several interacting environmental conditions such as temperature, vapor pressure deficit, nutrition and water availability (Kaiser et al., 2015; Kirschbaum, 2004). Although growth under drought stress is limited by many physiological mechanisms that interact into complex feedback cycles involving E_{plant}, A_{net}, and plant hydraulics (Tardieu and Parent, 2017), allocation to new root growth seems to ameliorate environmental stress in shallow-rooted seedlings. Furthermore, the architecture of newly developed roots, including traits such as rooting depth, fibrosity, and specific root length (SRL), among others, is key to the water uptake capacity of seedlings (Moler et al., 2022; York et al., 2013), which could explain part of the variation in the relationship between new root allocation, E_{plant}, and A_{net} (Fig. 4). An increase in E_{plant} and A_{net} can further promote root development since the main source of carbon for early root establishment in species like Douglas-fir and Sitka spruce (*Picea sitchensis*) are the newly produced photosynthates (van den Driessche, 1987). Root and foliage allocation may also depend on the isohydric or anisohydric water use strategies of each species (Sade et al., 2012). Our three species exhibit different degrees of isohydric behavior (Baker et al., 2019; Loewenstein and Pallardy, 1998). This conservative water use behavior could explain the positive relationship between root allocation and gas exchange since more access to water would mitigate the limitations to gas exchange by the restricted stomatal conductance. In the absence of sufficient root development, prolonged drought stress can limit growth due to low gas exchange and ultimately cause carbon starvation and hydraulic failure (McDowell, 2011).

5. Conclusions

Our results suggest that drought memory impacts the ability of tree seedlings to establish and acclimate in the face of successive droughts, thereby affecting the capacity of forests to regenerate and respond to harsh environmental conditions and climate change. Biomass allocation to new roots was positively correlated with both whole seedling transpiration and net photosynthesis rates in all species during the early establishment phase. However, biomass allocation to new roots and foliage in response to previous drought exposure varied across species. Drought primed western larch seedlings exhibited increased biomass allocation to new roots, which coincided with increased water uptake capacity and transpiration rates. In contrast, Douglas-fir seedlings exhibited earlier budbreak and increased biomass allocation to foliage in response to drought priming, indicating formation of a drought memory, which correlated with a higher photosynthetic rate. Finally, biomass allocation and gas exchange of black walnut seedlings exposed to drought were unaffected by previous drought exposure. These contrasting effects of drought priming across successive growing seasons suggest that the formation and effects of drought memory are closely tied to each species' unique ecophysiological traits and adaptive strategies for drought resistance.

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Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2025.106094.

Data availability

The datasets generated for this study are available on request from the corresponding author.

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