Temperate tree seedlings show extensive plastic response to water deficit and heat stress, but it is insufficient to fully mitigate decline in growth

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Abstract

Heat, cold, drought, salinity, oxidative stress, and nutrient deficiency are among the abiotic stressors that plants face because of climate change. However, these stressors do not occur in isolation, which increases plant vulnerability to pathogens and herbivory. While plants cannot quickly escape changes in air temperature and soil water availability, they still can acclimate to the new abiotic and biotic conditions in their current environments. Since temperate forests in Canada cover 9% of the world's total forest area (approximately 346 million hectares) and boreal forests store one-third of the world's terrestrial carbon, it is crucial to evaluate how heat and drought together impact the growth and functioning of native trees in these ecosystems. To support this effort, we conducted a greenhouse experiment exposing five native tree species to six different environmental treatments involving heat and drought, to assess specifically: 1) effects of water deficit and heat, alone and together, on seedling performance, 2) the plastic response of seedlings to water deficit and heat; and 3) the role of phenotypic plasticity to maintain plant performance under stress. We found, first, that growth is significantly reduced under water deficit, while warmer temperatures have a neutral to positive effect on growth, depending on the species (broadleaves versus conifers). Second, the effects of water deficit and heat on the phenotype are idiosyncratic among species, given that a) no single trait show a consistent response to the stressors imposed across all species; b) some species only showed a multivariate trait response to water deficit (i.e., *Acer saccharum,* and *Picea glauca*), others only to heat (i.e., *Betula alleghaniensis,* and *Pinus resinosa*) and only *Q. rubra* responded to both stressors; and c) a unique set of traits per species was associated with their responses to water deficit and/or heat. Our results illustrate the various and unique ways in which plant species are affected by and respond to environmental stress, highlighting the vulnerability of natural ecosystems to global warming.

Introduction

Climate change is leading to declines in plant performance due to the simultaneous occurrence of multiple interacting stressors (Pascual et al., 2022). Heat, cold, drought, salinity, oxidative stress, and nutrient deficiency are among the abiotic stressors that plants face because of climate change. However, these stressors do not occur in isolation, which increases plant vulnerability to pathogens and herbivory (Zhang et al., 2025). Additionally, the effects of each stressor can change in the presence of other stressors, making it challenging to reliably predict the effects of combined stressors based on their individual effects (Pirotta et al., 2022; Zandalinas et al., 2021). For example, a rise in air temperatures with similar precipitation regimes can still lead to drought stress due to higher evaporative demands, which decrease the water availability of the overall ecosystem (Gebrechorkos et al., 2025; Xu et al., 2023). Yet, we still do not fully understand the compound effects of heat and drought on tree performance.

While plants cannot quickly escape changes in air temperature and soil water availability, they still can acclimate, adapt, or migrate in response to those changes (Feeley et al., 2012). The long lifespan of tree species limits their capacity to adapt to new environmental conditions caused by climate change, and migration rates have been shown to be too slow when compared to the rate of warming (Hansson et al., 2021). Therefore, acclimating to the new abiotic and biotic conditions is essential to mitigate the effects of environmental stress on growth, reproduction, and survival (i.e., plant performance) (Feeley et al., 2023).

Plants acclimate by adjusting their anatomical, morphological, and physiological characteristics to maintain performance. These changes in ecophysiological characteristics optimize resource acquisition, conservation, and allocation under the non-optimal conditions. Theory shows that under limited resource availability (e.g., drought), we would expect phenotypes to become more conservative by investing in costly, long-lived leaves (high leaf mass per area (LMA), low nitrogen concentration, and low maximum photosynthetic rate) and reducing their stomatal conductance (gs) (I. J. Wright et al., 2004). Still, the entire (integrated) plant phenotype responds to the set of environmental conditions the plant is facing (Blonder et al., 2023; Díaz, 2025), and this response will be determined by the unique selective pressures imposed by all the stressors experienced (Luong & Loik, 2022).

Heat and drought are significant stressors, especially when they occur together, resulting in the largest crop yield losses, totaling approximately \$200 billion (Suzuki et al., 2014). These stressors are detected by different parts of the plant (Gebrechorkos et al., 2025). Additionally, plant responses to these stressors tend to oppose each other (Sato et al., 2024). When soil water availability decreases due to reduced rainfall, plants close their stomata to conserve water and prevent cavitation. Conversely, higher temperatures cause stomata to open, allowing the leaves to cool and avoid thermal damage from oxidative stress. As a result, the effect of heat and drought compound each other: increased temperatures lead to higher water use, while water deficits result in higher leaf surface temperatures (Sato et al., 2024).

Efforts have been made to understand the responses of trees to heat and drought individually. It has been shown that a widespread positive effect of warming on tree growth exists, with a stronger positive effect for species from higher latitudes (Li et al., 2020; Way & Oren, 2010). This could indicate that tree growth is not optimal in their current native environments, a hypothesis supported by the fact that evergreen species from boreal to tropical biomes have shown an upward shift of 0.34°C per °C in the thermal optimum of photosynthesis under warming conditions (Crous et al., 2022). Moreover, plants limited by cold temperatures showed under warming an increase in their maximum rate of carboxylation by Rubisco (VCmax), maximum photosynthesis electron transport rate (Jmax), maximum carbon assimilation per mass (Amass), and leaf stomatal conductance (gs) (Crous et al., 2022; Dai et al., 2021; Li et al., 2020). In contrast, plants exposed to temperatures above their thermal optimum of photosynthesis showed a reduction in their Amass, VCmax, Jmax, and chlorophyll concentration (CHL). Still, most tree species cooccurring in the same environment show idiosyncratic responses to warming (Li et al., 2020; G. Wu et al., 2018; T. Wu et al., 2019).

Tree mortality under water stress can be due to carbon starvation, hydraulic failure, and biotic agents that can amplify or be amplified by the other two mechanisms (McDowell et al., 2008). Species that have a tight control of their water loss through stomatal opening regulation (i.e., isohydric species) tend to die of carbon starvation while those that keep moving water despite soil water reduction (i.e., anysohidric species) tend to suffer from hidraulic failure (McDowell et al., 2008). It has been observed that isohydric species under severe water stress reduce their gs and Amass, however their root carbon reserves increase (Galvez et al., 2011). Additionally, under moderate water stress trees try to maintain their aboveground growth and access to resources, while under server stress, a higher investment to root growth results in an increase in their root mass fraction (Brunner et al., 2015). Therefore, drought in contrast to heat results in plant growth reduction or stagnation, a fact observed in cities where extreme heat events during the summer can result in a reduction of tree growth by 64% (Marchin et al., 2025).

North American boreal forests exhibit a significant west-east gradient in water availability and are projected to experience the lowest climatic moisture deficits in the future (D'Orangeville et al., 2016). Therefore, these regions have been proposed as a refuge for boreal species highly susceptible to drier conditions. However, there has been a lack of evidence of boreal tree expansion toward northern locations (Rotbarth et al., 2023). And, contrary to expectations, the temperate broadleaf forest is not migrating northward as quickly as was initially anticipated due to dispersal and demographic constraints (Rotbarth et al., 2023; Vissault et al., 2020). Thus, the maintenance of current populations in boreal and temperate forests will depend on individuals' ability to acclimate to warmer and drier conditions where they occur.

This project examines the response of Canadian temperate seedlings to heat, drought, and their combined effects. Temperate and boreal forests in North America are expected to experience higher summer temperatures, accompanied by either similar or lower levels of precipitation (Cohen et al., 2019). Future climate projections indicate that temperatures in Canada are expected to increase by 2 to 7 °C, depending on the effectiveness of efforts to reduce greenhouse gas emissions (Cohen et al., 2019). Additionally, these projected changes will compound the already observed rise in average annual temperature of over 1 °C between 1900 and 2016 (Cohen et al., 2019). Since temperate forests in Canada cover 9% of the world's total forest area (approximately 346 million hectares) and boreal forests store one-third of the world's terrestrial carbon (FAO, 2020), it is crucial to evaluate how heat and drought together impact the growth and functioning of native trees in these ecosystems. To support this effort, we conducted a greenhouse experiment to evaluate the effects of warming and water deficit, both individually and in combination, on the phenotype and performance of temperate tree seedlings. Specifically, by exposing five native tree species to six different environmental treatments involving heat and drought, we asked the following questions: 1) What are the effects of water deficit and heat, alone and together, on seedling performance? 2) What is the plastic response of seedlings to water deficit and heat? and 3) Does phenotypic plasticity allow seedlings to maintain their performance under stress? We expected that the compound effect of heat and drought would result in the strongest reduction in plant performance (i.e., growth).

Methodology

Experimental Design

We assessed the effects of water deficit and rising temperatures on five native Canadian tree species in a greenhouse at the University of Waterloo (Waterloo, ON, Canada). Using a factorial design, the trees were exposed to three water levels (low- (LWD), medium- (MWD), and high-water deficit (HWD)), and two temperature settings (ambient greenhouse (AMBIENT) and warmer temperatures with an open-top chamber (WARMED)). Trees were randomly assigned to one of 10 tables, and tables were rotated biweekly. The experiment ran from June 1st to September 20th, 2021. To mimic the natural decline of soil water due to drought, we first placed all the trees in LWD for a week. Then, we moved the trees assigned to MWD and HWD to the MWD treatment. After a week, the HWD trees were also transferred to this treatment. The experiment involved 180 saplings from species commonly used in forestry, namely *Acer saccharum* (ACSA), *Betula alleghaniensis* (BEAL), *Picea glauca* (PIGL), *Pinus resinosa* (PIRE), and *Quercus rubra* (QURU). Six seedlings of each of the five species were grown in each of the six treatments. The trees were obtained as 1 year-old seedlings in tube stock from the Pépinière et centre de semences forestières de Berthier of the Ministère des Forêts, de la Faune et des Parcs, and grown in circular pots containing a potting mix (80% Canadian sphagnum peat moss: 20% coarse perlite).

To expose trees to water deficit and warmer temperatures, we used the "Snow and Tinger" system and open-top chambers, respectively (Figure 1). The "Snow and Tiger" system uses capillarity irrigation to control the pots' soil water content (Fernández & Reynolds, 2000; Marchin et al., 2020; Snow & Tingey, 1985). This maintains a constant water potential and water deficit across all pots in a given treatment over the duration of the experiment, irrespective of their overall size, root size, or properties (Fernández & Reynolds, 2000; Lambrecht et al., 2007; Marchin et al., 2020). In each water treatment, half of the plants from each species was passively warmed inside an open-top chamber. We used open-top chambers with lids, custom-built to fit around individual trees using 2-mm-thick, UV-transmissive plexiglass, with a 13 cm hole in the lid. Open-top chambers are an affordable and effective tool for simulating warming climate conditions by capturing solar radiation that passively warms the inside air and soil while allowing natural light levels and gas exchange (De Frenne et al., 2013; Welshofer et al., 2018).

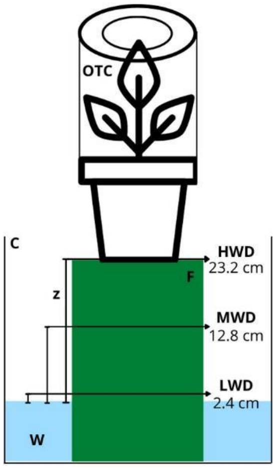

Figure 1. Diagram of the water deficit method, including the open-top chamber to increase the air and soil temperature. Pots (height: 17 cm, diameter: 20.32cm, volume: 3.8L) were placed on top of a column of material with low water permeability (F) (here, floral foam: 22.4 cm x 7.6 cm x 10.4 cm). The distance (z) between the water table (W) and the bottom of the pot determines the degree of water deficit (LWD: low water deficit, MDW: medium water deficit, HWD: high water deficit) (Adapted from Marchin et al. (2020)). W was held constant, and z was varied among treatments. Based on a pilot project ran during the summer of 2020, we used the following three levels for z: 2.4 cm for LWD, 12.8 cm for MWD, and 23.2 cm for HWD.

Environmental conditions

We measured the environmental conditions imposed on the plants by using 90 Flower Care™ sensors. Fifty-four sensors were placed within pots with open-top chambers and 36 in pots without open-top chambers. The sensors recorded air temperature (°C, AirTemp) and soil humidity (%, SH) every hour during the experiment. Additionally, we used a Teros 12 Soil Moisture Sensor to measure the volumetric water content (m^3/m^3, VWC), temperature (°C, SoilTemp), and bulk electrical conductivity (dS/m, BD) of each of the pots at least once per month.

Ecophysiological Traits

To assess the response of trees to water deficit and warming, we measured 37 functional traits from the leaves, stem, roots, and the whole plant, following standard procedures (Pérez-Harguindeguy et al., 2013). See the full details in the supplementary material. We focused on assessing key physiological functions expected to be affected by water deficit and heat, and to play a role in maintaining plant performance under these stresses (Table 1). These are resource acquisition (RA), resource conservation (RC, water transport (WT), temperature regulation (TR), photoprotection (PP), a specific type of resource conservation, mechanical support (MS), resource storage (RS), and biomass allocation (BA).

Table 1. Traits measured, abbreviations, units, and associated physiological function: resource acquisition (RA), resource storage (RS), water transport (WT), temperature regulation (TR), photoprotection (PP), mechanical support (MS), biomass allocation (BA). ‡Traits measured only on broadleaf species. †Traits measured only on individuals assigned to the low water deficit + ambient temperature and high-water deficit + heat treatments.

Traits	Abbr.	Units	Putative functional role
Leaf traits			
Stomatal width‡	SW	µm	RA - WT
Stomatal pore Index‡	SPI	%	RA - WT
Leaf thickness	LT	mm	RA – RS – WT – MS
Leaf mass per area	LMA	$g\ m^{-2}$	RA – RS – WT – MS
Leaf water potential at turgor loss point†	ψTLP	MPa	RA – RS – WT
Osmotic potential at full turgor†	ψ100	MPa	RA – RS – WT
Relative water content at turgor loss point†	RWC_{TLP}	%	RA – RS – WT
Modulus of elasticity†	ε	%	MS
Predawn water potential‡	PDwp	MPa	RA – WT
Delta water potential‡	Dwp	MPa	RA – RS – WT
Leaf temperature differential	LTD	°C	TR
Linear electron flow	LEF	unitless	RA - PP
Non-photochemical quenching estimated	NPQt	unitless	PP
Quantum Yield of Photosystem II	Phi2	unitless	RA
Ratio of incoming light lost via non-regulated processes	PhiNO	unitless	PP
Chlorophyll concentration	CHL	$mg\ m^{-2}$	RA
Maximum carbon assimilation per mass	Amass	$\mu mol\ CO_2\ g^{-1}\ s^{-1}$	RA
Stomatal conductance	gs	$mol\ H_2O\ m^{-2}\ s^{-1}$	RA – RS
Intrinsic water use efficiency	WUEi	$\mu mol\ CO_2\ mol^{-1}\ H_2O$	RA – RS
Leaf carbon concentration	LCC	%	RA – RS
Leaf nitrogen concentration	LNC	%	
Stem traits			
Hubber value	HV	$m^2\ m^{-2}$	WT – BA
Stem specific density	SSD	$g\ cm^{-3}$	RS – WT – MS
Fine (or Absorptive) Root Traits			
Root dry matter content	RDMC	$g\ g^{-1}$	RS
Specific root length	SRL	$m\ g^{-1}$	RA
Average root diameter	ARD	mm	RA – RS
Root carbon concentration	RCC	%	RS
Root nitrogen concentration	RNC	%	RA
Biomass Allocation			
Leaf mass fraction	LMF	$g\ g^{-1}$	BA
Root mass fraction	RMF	$g\ g^{-1}$	BA

Leaf traits

At the leaf level, we studied 21 functional traits (Table 1). All these traits were measured in at least one young, fully developed leaf, following the standard procedures explained below, and two months after the plants were established in their respective environments, allowing them to acclimate to their environmental conditions (August 9-26, 2021).

We measured two anatomical and two morphological leaf traits. Stomatal width (SW), and pore index (SPI) are anatomical traits that contribute to regulating plant water loss (Buckley, 2019). The stomata pore index is an integrative trait that combines both stomatal density and size to reflect the leaf's stomatal conductance, and it is calculated as: $SPI = stomatal\ density \times stomatal\ length^2 \times 10^{-4}$ **(Eq. 1).** Leaf mass per area (LMA) and leaf thickness (LT) are two morphological traits part of the leaf economic spectrum, a trade-off between strategies of long life span with low nitrogen content (resource conservation) and fast turnover with high nutrient concentration (resource acquisition), and therefore are associated with the plant's photosynthetic capacity, resource-use strategy, and water transport: (Díaz et al., 2016; I. J. Wright et al., 2004).

The capacity of plants to regulate their hydric status was studied by measuring the predawn water potential (PDwp, 3:00 – 5:00 h) and the daily change in water potential (Dwp = MDwp – PDwp) using a Scholander Pressure Chamber (Model 1505D-EXP/PMS- Instrument-Albany, OR) (Choat et al., 2012; Williams & Araujo, 2002).

Leaf cost production and photosynthetic capacity were assessed by measuring leaf carbon and nitrogen concentration (LCC and LNC), as well as chlorophyll concentration (CHL). Carbon and nitrogen concentrations were measured using combustion conversion of 0.9-1.0 mg of ground sample material to gas through a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) at the Environmental Isotope Lab of the University of Waterloo. Chlorophyll concentration was measured using a chlorophyll content meter capable of handling small needles (CCM-300, Opti-Sciences, Hudson, USA).

Maximum carbon assimilation per mass (Amass), stomatal conductance (gs), and intrinsic water use efficiency (iWUE) were measured from 10:00 to 12:00 h on a young, fully developed leaf using a LiCor 6800 (Li-Cor, Lincoln, NE, USA). Photoprotective mechanisms allow plants to dissipate the excess light energy they cannot use under stress (Derks et al., 2015). From 10:00 – 12:00 h on each of three fully developed leaves, we measured the amount of energy used towards photosynthesis (Phi2 – photochemistry) and lost via non-regulated processes (PhiNO – fluorescence) using the MultispeQ v2.0 (PhotosynQ, USA). From the MultispeQ v2.0, we also obtained the linear electron flow that is a proxy of photosynthesis (LEF), a calculated non-photochemical quenching (NPQt), and a leaf temperature differential which is the difference between ambient and leaf temperature (LTD).

To characterize the drought stress tolerance of the study species (Bartlett et al., 2012), we performed pressure-volume (PV) curves for individuals in the LWD/ambient and HWD/warmed treatments. From the PV curves, we extracted four hydraulic traits: turgor loss point (ψTLP), the osmotic potential at full turgor (ψ_{100}), relative water content at the turgor loss point (RWC_{TLP}), and modulus of elasticity of the cell walls (ε).

Stem traits

At the stem level, we studied the stem-specific density (SSD, mg mm^{-3}) and Huber value (HV), two morphological and physiological traits related to water transport. SSD affects the four physiological functions of the xylem: water transport safety, water transport efficiency, plant support, and resource storage (Chave et al., 2009; Pratt & Jacobsen, 2017). The Huber value

(HV, m^2 m^{-2}) is an integrative trait that relates the sapwood area ratio of a stem or branch (the fraction of xylem that conducts sap) to the total leaf area it supplies. It thus provides a water supply to demand ratio that has been shown to scale with plant stature and wood density (Mencuccini et al., 2019).

Root traits

We studied five fine-root traits involved in resource acquisition and conservation (Bergmann et al., 2020; Freschet, Roumet, et al., 2021). After tree harvesting, roots were manually washed in water until all soil was cleared. Then, we collected at least ten fine absorptive root samples per individual, defined here as 1st to 3rd-order roots for all species (Freschet, Pagès, et al., 2021; McCormack et al., 2015). On these fine roots, we measured specific root length (SRL, cm mg^{-1}), mean root diameter (RD, cm), root dry matter content (RDMC), root nitrogen content (RNC), and root carbon content (RCC).

Biomass allocation traits

At the end of the experiment, leaf mass fraction (LMF) and root mass fraction (RMF) were measured as the ratio of total leaf dry mass and root dry mass to total plant dry mass, respectively. All plants were harvested between September 16 and 21. The leaves, stems, and roots were separated and weighed after being oven-dried for 72 h at 65 °C. Leaves that were naturally shed before the harvest date were collected, dried, and included in the total leaf mass measurements. The weight of the fine roots collected for root trait measurements was added to the total root biomass.

Plant Performance

We measured plant performance as their relative growth rate (RGR) based on total biomass. To calculate the initial total biomass of the plants without killing them, we measured the total pot weight after watering the plants and letting the pots drain for 20 mins. This weight combines plant weight and soil weight at field capacity. At the end of the experiment, we repeated this process to obtain the final weight at field capacity. RGR was calculated as:

$$RGR = \frac{Ln(Final\ Weight) - Ln(Initial\ Weight)}{Ln(Initial\ Weight)} \qquad \textbf{Eq. 1}$$

This method assumes that soil maintains its water-holding capacity throughout the experiment. Three individuals with negative growth values were removed from all analyses using RGR.

Statistical Analyses

All the statistical analyses were performed in R v4.2. The models' assumptions were verified using validation plots and statistical analyses (Zuur et al., 2010). The mean of each treatment is reported for all the statistical analyses showing a significant effect. All the analyses were performed for each species individually.

To evaluate the individual and combined effects of water deficit and heat on plant performance (Question 1), we built a linear model predicting RGR as a function of the water treatment, heat treatment, and their interaction (package {stats}, function (lm)). The interaction term between water deficit and heat was reported when statistically significant. For each species, differences among treatments were identified using post-hoc Tuckey tests.

To evaluate the plastic response of individual traits to water deficit and heat (Objective 2), we used linear and mixed models predicting trait values as a function of the water treatment, heat treatment, and their interaction. Linear models were used for those traits we measured once during the entire course of the experiment (package{stats}, function(lm)). Linear mixed models were used for those traits that were measured over multiple days, with the day of measurement as a random effect (packages {lmerTest}, functions (lmer)). The interaction term between water deficit and heat was reported when statistically significant. To evaluate the multivariate trait response to water deficit and heat, we performed a redundancy analysis with water deficit and heat as the explanatory variables (package {vegan}, function (rda)). For the RDA analysis, we removed highly correlated functional traits (Pearson correlation coefficient higher than or equal to 0.7).

To evaluate whether trait plasticity contributed to RGR maintenance under water deficit and heat (Objective 3), we performed stepwise model selection in both directions (using the package {stats}, function(step)) for a multiple linear regression that predicted a decrease in RGR based on changes in functional traits. We calculated the relative changes in functional trait values and relative growth rate under medium and high-water deficit (MWD and HWD) compared to LWD, and under warmer conditions (WARMED) for heat, since there was no difference in RGR between medium and high levels. The change in functional trait values was calculated as follows:

$$|\Delta FT| = \left|\frac{FT_{control} - FTi_{stressed}}{\underline{FT_{control}}}\right| \qquad \textbf{Eq. 3}$$

$\underline{FT_{control)}}$ represents the mean trait value under low water deficit or greenhouse temperatures and $FTi_{stressed}$ represents the observed trait value for every individual in the medium and high-water deficit or warmer temperatures, with samples sizes of n=24 for water deficit (except for *P. glauca* where n = 23) and n=18 for heat. The change in relative growth rate was calculated as follows:

$$\Delta RGR = \frac{\underline{RGR_{control}} - RGRi_{stressed}}{\underline{RGR_{control}}} \qquad \textbf{Eq. 4}$$

$\underline{RGR_{control}}$ represents the mean relative growth rate under low water deficit or greenhouse temperatures and $RGRi_{stressed}$ the observed relative growth rate for every individual in the medium and high-water deficit or warmer temperatures. Given equation 4, positive ΔRGR values indicate declines in growth rate. Since the control environment is used as reference for changes in growth and, negative correlations between ΔRGR and |ΔFT| indicate that large changes in trait are associated with small declines in RGR, thus maintaining performance. In contrast, positive correlations indicate that changes in traits are associated with large decreases in RGR.

We included in the full model all significant traits in either univariate or multivariate analyses from objective 2. The selected model was the one with the lowest AIC and the fewest traits. Before running the stepwise selection, highly correlated functional traits were removed (Pearson correlation coefficient of 0.7 or higher for all species). We confirmed that the predictors in the best model had a variance inflation factor (VIF) below 3 (Zuur et al., 2010, using {car}, function(vif)). When the best model contained predictors with VIF values exceeding 3, those predictors were removed one by one from the full model, starting with the predictor with the highest VIF, until all predictors in the best model had VIF values below 3.

Results

Water deficit and heat treatments were effective

The watering treatments successfully resulted in three water deficit levels. Treatments had significantly different volumetric water contents (μ_{LWD} = 38.2%, μ_{MWD} = 35.7%, μ_{HWD} = 31.3%, p = 2.2e-15, Figure S1). Open-top chambers resulted in a significant increase of 0.54 °C in average daily temperature ($F_{(1,83)}$ = 17.86, $p < 0.0001$), with the most pronounced warming occurring during the morning (6h00 – 13h00: + 0.60°C) and nighttime (20h00 – 6h00: + 0.64°C). See Figure S2).

Water deficit and heat reduced plant performance (RGR)

All species showed a reduction in RGR with water deficit, both in ambient and heated treatments (Figure 2). For broadleaf species, RGR in the MWD and HWD treatments were similar and were 36 to 55% lower than in the LWD treatment (*A. saccharum*: $F_{(2,32)}$ = 19.83, $p < 0.0001$; *B. alleghaniensis:* $F_{(2,32)}$ = 12, $p < 0.001$; *Q. rubra:* $F_{(2,32)}$ = 18.4, $p < 0.0001$). Warmer temperatures did not affect the RGR of broadleaf species. For coniferous species, RGR in HWD was 26 to 45% lower than in the LWD treatment (*P. glauca*: $F_{(2,31)}$ = 5.86, $p < 0.01$; *P. resinosa:* $F_{(2,30)}$ = 7.23, $p < 0.01$) and RGR in the MWD treatment was not different from either LWD or HWD. Additionally, warmer temperatures led to higher values of RGR for *P. glauca* but did not affect *P. resinosa* (*P. glauca*: $F_{(1,31)}$ = 4.09, $p = 0.05$). In none of the species did water deficit and temperature interact to affect RGR.

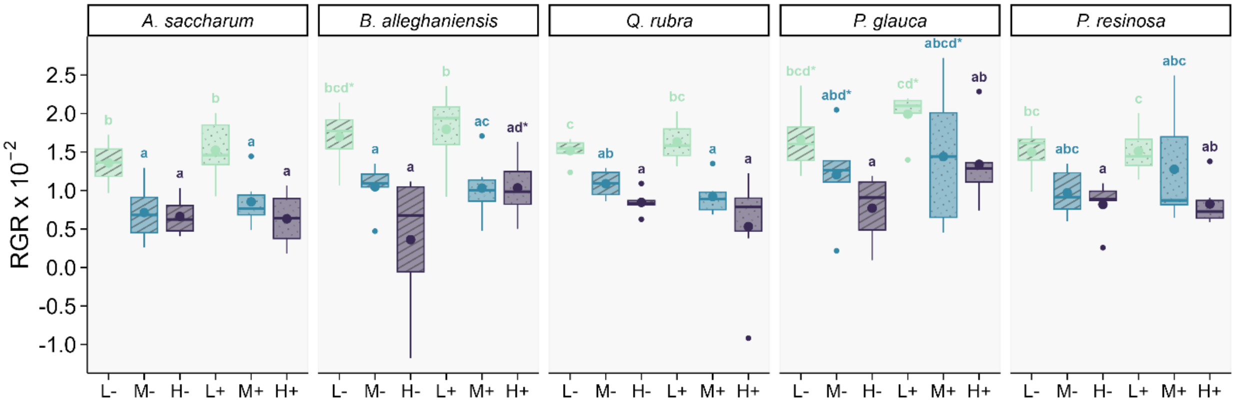

Figure 2. Relative growth rate (RGR) for each species under six different water deficit and heat treatments (n = 6). L, M, and H, followed by a minus (–) sign, are the low, medium, and high-water deficit treatments without heat treatment, while L, M, and H, followed by a plus (+) sign, are the low, medium, and high-water deficit treatments with heat treatment. Different letters denote statistically significant differences among the 6 treatments. Similar letters followed by a * indicate that the differences among the treatments are marginally significant ($0.05 > p \leq 0.10$). Lines in the box plots indicate the median values, whereas solid dots indicate the mean values.

Water deficit and heat have different effects on the phenotype.

Univariate and multivariate analyses of the effects of water deficit and heat on plant functional traits showed that 1) different species responded differently to the stressors, and 2) for each species, the traits that responded to heat differed from the traits that responded to water deficit (Table 2 and Figure 3). No single trait changed in all species in response to heat or water deficit, however, leaf thickness (LT) showed a plastic response under water deficit conditions for all species but *B. alleghaniensis* (*A. saccharum:* $F_{(2,32)} = 3.6$, $p = 3.9\text{e-}2$; *Q. rubra:* $F_{(2,32)} = 7.3$, $p = 0.01$; *P. glauca* $F_{(2,31)} = 3.4$, $p = 4.5\text{e-}2$, *P. resinosa:* $F_{(2,30)} = 2.8$, $p = 7.5\text{e-}2$). Additionally, *A. saccharum, B. alleghaniensis,* and *P. resinosa* showed a strong increase in leaf temperature differential (LTD, a measure of cooling), under warmer conditions: cooling increasing from ca. 40 to ca. 90% (*A. saccharum:* $F_{(1,32)} = 8.8$, $p = 5.7\text{e-}3$; *B. alleghaniensis:* $F_{(1,32)} = 8.2$, $p = 7.4\text{e-}3$; *P. resinosa:* $F_{(1,30)} = 7.7$, $p = 9.5\text{e-}3$). *B. alleghaniensis* showed the fewest changes in trait values in response to the two stressors, with no plastic response to water deficit. In all species, the response of a few traits to one stress was contingent on the other stress (Table 2, water deficit and heat interaction).

Table 2. Summary table of functional traits that responded to water deficit, heat, or both. The "Combined effect of Water Deficit + Heat" row corresponds to those traits measured only in individuals in the LWD- and HWD+ treatments and, therefore, whose trait response cannot be ascribed to an individual stressor. Values in parentheses indicate the percentage and direction of trait change in high or medium water deficit relative to low water deficit for water deficit, trait change in the warm treatment relative to ambient treatment for heat, and trait change under high water deficit and warmed temperature relative to low water deficit and ambient temperature for the combination of water deficit and heat. All traits reported changed significantly in response to the stresses imposed ($p \leq 0.05$), except those followed by a dot (•), which changed marginally ($0.5 > P \leq 0.1$). See Table 1 for trait acronyms.

Treatment / Species	*A. saccharum*	*B. alleghaniensis*	*Q. rubra*	*P. glauca*	*P. resinosa*
Water Deficit	RDMC (+28.5) SPI (+18.6) LT (-8.7) SSD (-14.3)		HV (+24.8) SSD (+22.1) RMF (+18.9)• LT (+12)• Ammass (-15.4)• RNC (-17.3)• Dwp (-42.7)	RDMC (+25.1) LMA (+19.4) LT (+17.2) RMF (+15.7) •	LCC (+1.31) LT (-9.9)• RDMC (-11) Dwp (-41.5)
Heat	LTD (+93.1) SPI (+9.2) • SW (-3.2) •	LTD (+40) PhiNO (+4.6) RMF (-6.9) NPQt (-18.4)	SPI (+13.7) RDMC (-13.7) RMF (-11) WUEi (-14.0)	gs (+31) • ARD (+14.8)• CHL (-9.9) WUEi (-9.9)	LNC(+83) LTD (+55.5) NPQt (+43.6) • ARD (+12.7) LMF (+3.7)• SSD (-3.4) RMF (-12.8) SRL (-16) • Amass (-33.1) gs (-35.7)•
Water Deficit and Heat Interaction	LT• Dwp LMA	SSD RMF	LT SSD • SPI •	LT SRL ARD	LT RDMC RNC
Combined effect of Water Deficit + Heat	RWC_{TLP} (-7.3%)		ε (+68%) ψ_{100} (-34.6%)		

All species exhibited a multivariate trait response to either heat or water deficit, with only *Q. rubra* showing a multivariate trait response to both stressors (Heat: *B. alleghaniensis* $F_{1,34} = 1.58, p = 0.048$, *Q. rubra* $F_{1,32} = 1.98, p = 0.009$, and *P. resinosa* $F_{1,32} = 2.57, p = 0.003$. Water deficit: *A. saccharum* $F_{2,33} = 1.49, p = 0.05$, *Q. rubra* $F_{2,32} = 1.34, p = 0.084$, and *P. glauca* $F_{2,32} = 1.38, p = 0.083$; Figure 3). For *Q. rubra,* together heat and water deficit explained 4.5 % of the variation in trait values *(p = 0.007)*. Heat explained 1.6% of the variation in trait values for *B. alleghaniensis (p = 0.048),* and 4.5% for *P. resinosa (p = 0.003)*. Water deficit explained 2.7% of the variation in trait values for *A. saccharum* (*p = 0.05*), and 2.2% for *P. glauca (p = 0.083)*. Different sets of traits were associated with the significant RDA 1 axis of each species (*A. saccharum* $F_{1,33} = 2.24, p = 0.016$, *B. alleghaniensis* $F_{1,34} = 1.58, p = 0.032$, *Q. rubra* $F_{1,32} = 2.12, p = 0.055$, *P. glauca* $F_{1,32} = 1.91, p = 0.07$, and *P. resinosa* $F_{1,32} = 2.57, p = 0.002$). The only similarities are that RMF decreases with heat in both *Q. rubra* and *P. resinosa* and increases with water deficit in *Q. rubra* and *P. glauca*. RDMC increases with water deficit in *P. glauca* and *A. saccharum*.

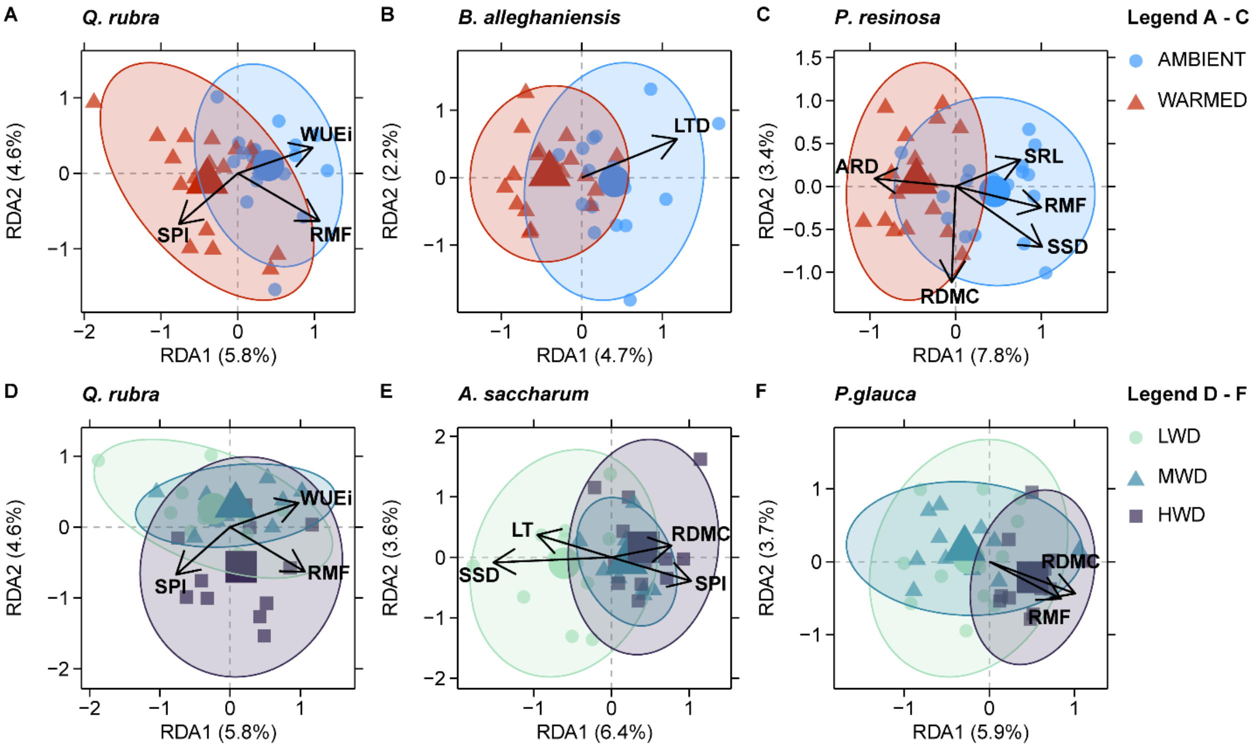

Figure 3. RDA plots for those species with a significant redundancy analysis of the effect of water deficit and heat. For clarity and readability, we presented the treatments separately, even in *Q.rubra*, for which both treatments had a significant effect.

Few functional traits showed adaptive plasticity, mitigating the effects of water deficit and heat on RGR

A. saccharum, *Q. rubra, P.* glauca, and *P.* resinosa showed changes in functional trait values under medium and high-water deficits that contributed to maintaining relative growth rate to levels similar to those under low water deficit (Figure 4A-D). For *A. saccharum*, *Q. rubra,* and *P.* glauca, trait plasticity mitigated declines in RGR under warmer conditions (Figure 6E-G). Stomatal width (SW), root mass fraction (RMF), leaf thickness (LT), and average root diameter (ARD) were the individual traits that contributed to RGR maintenance under water deficit for *A. saccharum* ($R^2_{adj} = 42\%, F_{3,20} = 6.65, p = 0.003$), *Q. rubra* ($R^2_{adj} = 30\%, F_{4,19} = 2.9, p = 0.05$), *P. glauca* ($R^2_{adj} = 36\%, F_{3,19} = 3.6, p = 0.03$) and *P. resinosa* ($R^2_{adj} = 30\%, F_{4,19} = 3.4, p = 0.03$)*,* respectively. The plastic response of midday and predawn water potentials (Dwp), intrinsic water use efficiency (WUEi), and leaf mass per area (LMA) were the individual traits that mitigated decline in RGR under warmer conditions for *A. saccharum* ($R^2_{adj} = 14\%, F_{1,16} = 3.8, p = 0.07$), *Q. rubra* ($R^2_{adj} = 27\%, F_{3,14} = 3.1, p = 0.06$), and *P. glauca* ($R^2_{adj} = 50\%, F_{2,15} = 9.7, p = 2e\text{-}3$), respectively. In *B. alleghaniensis*, no plastic changes in trait values mitigated the decline in RGR under either water deficit or warmer conditions. A few plastic responses contributed to the decline in performance of the species: under water deficit shifts in LMA led to larger declines in growth in *A. saccharum*, shifts in HV led to larger declines in growth in *Q.*

rubra, and shifts in LNC led to larger declines in growth in *P. resinosa*. Under heat stress, shifts in Amass led to larger declines in growth in *Q.rubra*.

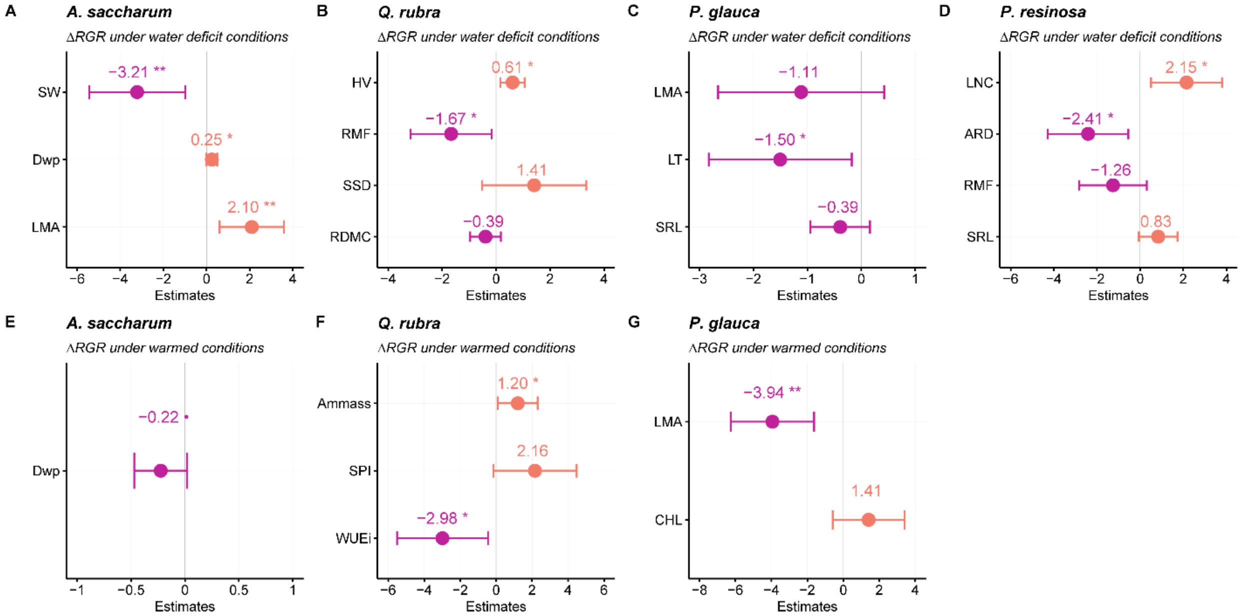

Figure 4. Forest plot of the significant multiple linear regression best model for each species under water deficit conditions (A – D) and warmed conditions (E – G).• = $0.5 > p \leq 0.01$, * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. Bold traits are those that significantly contribute towards RGR maintenance under stressful conditions.

Discussion

Our study examined the response to water deficit and heat stress in saplings from five native tree species found in temperate and boreal forests of North America. We found, first, that growth is significantly reduced under water deficit, while warmer temperatures have a neutral to positive effect on growth, depending on the species (broadleaves versus conifers). Second, the effects of water deficit and heat on the phenotype are idiosyncratic among species, given that a) no single trait show a consistent response to the stressors imposed across all species; b) some species only showed a multivariate trait response to water deficit (i.e., *Acer saccharum,* and *Picea glauca*), others only to heat (i.e., *Betula alleghaniensis,* and *Pinus resinosa*) and only *Q. rubra* responded to both stressors; and c) a unique set of traits per species was associated with their responses to water deficit and/or heat. Finally, in only a handful of traits was the plastic response to the stressor adaptive (able to maintain the decline in growth). Collectively, these results illustrate the various and unique ways in which plant species are affected by and respond to environmental stress, highlighting the vulnerability of natural ecosystems to global warming.

Water deficit reduced plant performance (RGR)

We anticipated reduced growth under conditions of heat and water deficit, both in isolation and in combination, as resource-poor environments induce physical stress. This stress triggers biochemical changes that impact metabolic and developmental processes. As expected, we observed a reduction in the relative growth rate under medium and high-water deficits compared to the low water deficit; however, the growth rates in medium and high-water deficits did not differ significantly. This was unexpected given that the high-water deficit reduced the volumetric water content by twice the amount of the medium water deficit (6.9% v. 2.5%). In contrast to water deficit, warmer temperatures did not hinder plant growth. In fact, for white spruce (*P. glauca*), warmer temperatures even caused non-significant increases in growth across all soil moisture conditions. This lack of response to temperature may be due to the small increase in temperature caused by the open-top chambers (WARMED), as well as the geographical location and provenance of the species.

The OTCs did lead to an increase in air temperatures of +0.54 °C; however, this rise is still below the future climate projections, which suggest an increase of 1.5 to 3.5 °C by 2100. *In-situ* experiments have demonstrated that temperate tree seedlings, such as *A. saccharum, P. resinosa,* and *Q. rubra*, grow better at warmer temperatures (+1.7 and +3.4 °C), but this positive effect was found to be contingent on exposure to water deficit (J. A. Wright et al., 2018). In contrast, none of our temperate potted plants showed adverse effects from the warmer temperatures, and the combination of warmer and drier conditions did not significantly suppress growth when compared to the control treatment. Therefore, the positive effects of warmer temperatures on the photosynthesis and growth of temperate trees in cold-limited environments may not become evident until a minimum temperature increase is achieved or may only occur at lower temperatures.

In comparison to temperate species, white spruce—the only boreal tree—showed a positive response in relative growth rate (RGR) to warmer temperatures, regardless of water deficit conditions. These findings contradict the average plastic reduction of approximately 25% in growth for midwestern boreal individuals when exposed to predicted future temperatures (+1.7 and +3.4 °C) (J. A. Wright et al., 2018). Tree-ring data from populations across North America indicate that the responses of white spruce populations to increased temperatures and drier conditions will vary by location and by provenance (Hynes & Hamann, 2020; Lu et al., 2019). Furthermore, it has been found that populations from south-central Ontario and southwestern Quebec show better growth in northern environments compared to local populations (Lu et al., 2019).

Since our stock of trees originated from nurseries in Quebec, the observed neutral and potentially positive effects on RGR to warming conditions could be due to their inherent higher capacity to withstand the warmer temperatures to which they were exposed, and support the idea that northeastern North America could serve as a refuge for temperate and boreal forests (D'Orangeville et al., 2016). Or it could just be that these five species are already adapted to the temperature to which we exposed them.

Water deficit and heat have different effects on the phenotype

Univariate and multivariate analyses revealed that the effects of the studied stressors are highly species-specific. The plastic response of each species to warming and water deficit differed. Further, each species showed a distinct response to these two stressors (whether individual or combined). For example, leaf thickness increased under drier conditions for the northern red oak (*Q. rubra*) and white spruce (*P. glauca*), decreased for sugar maple (*A. saccharum*) and red pine (*P. resinosa*), and remained unchanged for yellow birch (*B. alleghaniensis*).

The northern red oak and white spruce exhibited changes in functional trait values under water deficit conditions, indicating a more conservative resource use. The northern red oak adopted a conservative strategy, characterized by greater investment in structural carbon and in the storage of carbohydrates and sugars in costly plant tissues. This was evident through its thicker leaves, denser stems, and increased root production under water deficit conditions. Additionally, it showed reduced stomatal opening at noon, resulting in similar predawn and midday leaf water potential values, associated with lower carbon assimilation. In contrast, white spruce did not show significant changes in carbon assimilation or water loss under drought conditions. However, like the northern red oak, it exhibited increased carbon investment and resource storage in expensive tissues, marked by thicker, denser needles and higher root production with lower water content.

On the other hand, sugar maple (*A. saccharum*) and red pine (*P. resinosa*) showed mixed responses: trait values shifted towards increased resource acquisition while others shifted towards increased resource conservation strategies under water scarcity. Sugar maple individuals under water deficit conditions had thinner leaves and less dense stems, which helped in faster resource acquisition. They also had a higher number of smaller stomata, allowing for improved carbon assimilation without significantly increasing water loss, along with root tissues that contained less water but needed higher carbon content. Red pine individuals exhibited thinner leaves and root tissues with high water content, indicating an acquisitive strategy. However, they also displayed a shift towards more conservative traits: needles with high carbon concentration that showed reduced stomatal opening at noon, resulting in similar predawn and midday leaf water potential values as in the low water deficit treatment.

Responses to warmer conditions, differed among broadleaves and conifers. Broadleaf species, such as sugar maple and northern red oak, showed an increase in the stomatal pore index, primarily associated with a rise in the number of stomata. Plants need to balance their carbon gain and water loss, and this balance is also influenced by the plant's need to cool its leaves under warmer conditions (Hofmann et al., 2025; Sato et al., 2024). Both increases and decreases in stomatal density have been reported under warmer conditions (Beerling & Chaloner, 1993; Ferris et al., 1996; Hill et al., 2014; Jumrani et al., 2017), each offering different benefits. In our specific experiment, and for these two species, an increase in stomatal density could help mitigate the effects of heat through transpiration-mediated cooling (Bertolino et al., 2019). This was the response of sugar maple, which, along with yellow birch, showed an increase of over 40% in their leaf temperature differential, indicating increased cooling. It is worth noting that for northern red oak, changes in SD were accompanied by a reduction in water-use efficiency, suggesting that the cooling advantage may come at the cost of higher water loss. This trade-off can be risky if higher temperatures lead to increased vapor pressure deficit (VPD), even with similar precipitation levels, as observed in the temperate and boreal forests of northeastern North America (Hofmann et al., 2025).

Conifer species responded to warmer conditions with opposite changes from each other in aboveground traits and similar changes in belowground traits. For aboveground traits, stomatal conductance increased in white spruce but decreased in red pine. The higher stomatal conductance in white spruce was linked to lower water use efficiency, which favored whole-plant growth over water conservation under warmer conditions. Conversely, red pine's reduced stomatal conductance was accompanied by decreased carbon assimilation and, counterintuitively, an increase in the leaf's cooling capacity. Enhanced leaf cooling in red pine was associated with an upregulation of controlled dissipation of excess light energy (higher NPQ), indicating that cuticle conductance might be a trait worthy of study for this species under different temperature regimes (Garen & Michaletz, 2025; Slot et al., 2021). Regarding belowground traits, both species exhibited an increase in average root diameter, possibly indicating an "outsourcing" strategy under warmer conditions due to fungi's ability to enhance plant stress tolerance (Laughlin et al., 2021; Yaffar et al., 2022).

The varied trait responses observed, coordination, and decoupling emphasize the importance of studying the integrated phenotype (Díaz, 2025). Our multivariate trait analysis revealed that whole-organism phenotypic responses align with the optimal partition theory. The optimal partition theory suggests that resource allocation is adjusted to maximize the capture of the most limiting resource under environmental stress (Bloom et al., 1985; Luong & Loik, 2022). Species that showed whole-organism responses to water deficit (*Q. rubra, A. saccharum*, and *P. glauca*) showed an increase in either or both root mass fraction and root dry matter content, supporting the allocation of resources towards root tissues that can increase the access to water. Still, species like the sugar maple have shown that thinner leaves can be favored in some species under water scarcity, indicating that different combinations of trait values can be successful in a certain environmental context (Blonder et al., 2023; Díaz, 2025). Another important takeaway is that the plastic response of different species to the same stressor can be vastly different, cautioning us against undue generalizations.

Few functional traits showed adaptive plasticity, resulting in limited mitigation pf the effects of water deficit and heat on RGR

Changes in stomatal width (*A. saccharum*), root mass fraction (*Q. rubra*), leaf thickness (*P. glauca*), and average root diameter (*P. resinosa*) values helped maintain RGR. These traits are associated with resource acquisition (SW, LT, and ARD), resource storage (LT and ARD), water transport (SW and LT), mechanical support (LT), and biomass allocation (RMF). Our results show that different species adjust different traits serving different ecophysiological functions to maintain growth under stress.

Reproduction, growth, and survival rates are the three fitness components (Violle et al., 2012). Seedlings can maximize their fitness by maintaining growth or survival. leading to demographic trade-offs within and across species (Ellis et al., 2021; Laughlin et al., 2020). The observed changes in plant phenotype favored whole-plant survival (growth-survival trade-off) as a response to drier and/or warmer environmental conditions (Díaz, 2025). Therefore, the few traits linked to growth maintenance under water scarcity may be because growth is not the primary performance component contributing to plant fitness in this system (Blonder et al., 2023; Laughlin et al., 2020). This is supported by the higher number of functional traits that changed under warmer conditions, the multivariate response to heat of *Q. rubra, B. alleganiensis*, and *P. resinosa*, and the roles of daily water regulation (*A. saccharum*), water use efficiency (*Q. rubra*), and leaf mass per area (P. glauca) in maintaining RGR (Blonder et al., 2023).

Furthermore, we found that some traits exhibit maladaptive plasticity in maintaining the relative growth rate under heat and water deficit conditions. Therefore, the observed adaptive plasticity was not enough to counteract the stressors' effects on the performance metric of interest (i.e., growth). Nevertheless, each species employed a distinct strategy to balance gains and losses, supporting both resource acquisition and conservation, which helped allocate carbon toward root tissue production and/or prevent thermal damage through cooling responses.

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