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Research paper

Short- and long-term responses of photosynthetic capacity to temperature in four boreal tree species in a free-air warming and rainfall manipulation experiment

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High latitude forests cope with considerable variation in moisture and temperature at multiple temporal scales. To assess how their photosynthetic physiology responds to short- and long-term temperature variation, we measured photosynthetic capacity for four tree species growing in an open-air experiment in the boreal-temperate ecotone 'Boreal Forest Warming at an Ecotone in Danger' (B4WarmED). The experiment factorially manipulated temperature above- and below-ground (ambient, +3.2 °C) and summer rainfall (ambient, 40% removal). We measured A/C_i curves at 18, 25 and 32 °C for individuals of two boreal (*Pinus banksiana Lamb.*, *Betula papyrifera Marsh.*) and two temperate species (*Pinus strobus L.*, *Acer rubrum L.*) experiencing the long-term warming and/or reduced-rainfall conditions induced by our experimental treatments. We calculated the apparent photosynthetic capacity descriptors $V_{Cmax,Ci}$ and $J_{max,Ci}$ and their ratio for each measurement temperate. We hypothesized that (i) $V_{Cmax,Ci}$ and $J_{max,Ci}$ would be down-regulated in plants experiencing longer term (e.g., weeks to months) warming and reduced rainfall (i.e., have lower values at a given measurement temperature), as is sometimes found in the literature, and that (ii) plants growing at warmer temperatures or from warmer ranges would show greater sensitivity (steeper slope) to short-term (minutes to hours) temperature variation. Neither hypothesis was supported as a general trend across the four species, as there was not a significant main effect (across species) of either warming or rainfall reduction on $V_{Cmax,Ci}$ and $J_{max,Ci}$. All species markedly increased $V_{Cmax,Ci}$ and $J_{max,Ci}$ (and decreased their ratio) with short-term increases in temperature (i.e., contrasting values at 18, 25 and 32 °C), and those responses were independent of long-term treatments and did not differ among species. The $J_{max,Ci}$:$V_{Cmax,Ci}$ ratio was, however, significantly lower across species in warmed and reduced rainfall treatments. Collectively, these results suggest that boreal trees possess considerable short-term plasticity that may allow homeostasis of $V_{Cmax,Ci}$ and $J_{max,Ci}$ to a longer term temperature treatment. Our results also caution against extrapolating results obtained under controlled and markedly contrasting temperature treatments to responses of photosynthetic parameters to more modest temperature changes expected in the near-term with climate warming in field conditions.

Keywords: A/C_i, acclimation, B4WarmED, boreal forest, growth temperature, J_{max}, measuring temperature, rainfall reduction, temperate forest, temporal scales, V_{Cmax}, warming.

Introduction

Adaptation and phenotypic plasticity are key mechanisms that plants use to attune themselves to changes in their environment on different time scales (hours, weeks, years; Matesanz et al. 2010, Nicotra et al. 2010, Valladares et al. 2014). Both are relevant to boreal and temperate species, which are adapted to considerable thermal and moisture variation and will experience considerable climate change this century. For long-lived taxa, phenotypic plasticity may be particularly important (Gunderson et al. 2000, Matesanz et al. 2010, Valladares et al. 2014), and given the role of photosynthesis in plant growth, acclimation of photosynthetic biochemistry to temperature variation on multiple time scales is important for understanding these ecosystems and their potential feedbacks to the carbon cycle.

Acclimation can be defined as any morphological or physiological adjustment of an individual plant to compensate for altered performance produced by an environmental change (Lambers et al. 2008). The acclimation of photosynthesis to changes in temperature is mainly related to changes in quantity and reaction rates of enzymes of the photosynthetic apparatus, and relative changes in the affinity of Rubisco for CO_2 and O_2 (Campbell et al. 2007, Lambers et al. 2008). In our conceptual framework, we follow Atkin and Tjoelker (2003), Smith and Dukes (2013) and Way and Yamori (2014) who identify several different kinds of acclimation (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). Type I acclimation refers to changes in the shape of the instantaneous response curve, whereas Type II acclimation involves changes in the base rate or intercept of the temperature response curve or elevation of the overall curve (Atkin and Tjoelker 2003, Smith and Dukes 2013). These are the main foci of our current work.

Neither of the two most widely used descriptors of photosynthetic capacity, $V_{C\max}$ (maximum carboxylation capacity) and $J_{\max}$ (maximum capacity of regeneration of RuBP-1,5), have shown consistent, predictable acclimation (of either Type I or II) to elevated growth temperatures, although Type II responses tend to either show down-regulation or no change (Kattge and Knorr 2007, Way and Yamori 2014). This lack of consistent response is problematic for land surface modeling. Additionally, the majority of reported experiments were conducted with large temperature differences among treatments, under controlled environmental conditions in greenhouses or growth chambers (Table 1 and Table S1 available as Supplementary Data at *Tree Physiology* Online). Thus, little prior research has been done under realistic field conditions, such as for plants experiencing longer term but more modest alterations in temperature, and we have little basis for knowing the applicability of prior work to such conditions. Moreover, it is common in models to apply conversion equations (commonly based on Arrhenius function) in order to adjust values to a standard and more easily comparable temperature, but some authors (Bernacchi et al. 2003, Scafaro et al. 2017) found systematic differences between the adjusted and measured values; herein, we conduct the same type of comparison using long-term field data.

Additionally, although joint variation in moisture availability and temperature is likely to be important for plants in many ecosystems (Stephenson 1990, Chapin et al. 1993, Choat et al. 2012), most studies have focused on either just temperature (Dreyer et al. 2001, Yamori et al. 2005, Hikosaka et al. 2006, Way and Sage 2008, Scafaro et al. 2017) or moisture (Maroco et al. 2002, Turnbull et al. 2002, Limousin et al. 2010, Sperlich et al. 2015, Zhou et al. 2016). Similar to responses to temperature, responses of $V_{C\max}$ and $J_{\max}$ to moisture availability have varied from up-regulation to down-regulation to no adjustment (Turnbull et al. 2002, Peña-Rojas et al. 2004, Mokotedi 2010, Albert et al. 2011, Galle et al. 2011, Ge et al. 2012, Gu et al. 2012, Yu et al. 2012, Cano et al. 2014, Kelly et al. 2016).

In an attempt to help fill knowledge gaps for high latitude forest species, relevant to the above issues, we assayed response of photosynthetic biochemistry to short-term temperature variation for four species growing under both long-term warming and rainfall manipulation conditions, using a realistic open-air experiment in Cloquet, MN, USA (Reich et al. 2018). The most common way to study this is using the Farquhar–von Caemmerer–Berry (FvCB) biochemical model of photosynthetic CO_2 assimilation in C_3 species (Farquhar et al. 1980), which estimates whether the limiting pathway to photosynthesis is imposed by the carboxylation capacity of the Rubisco or the regeneration of RuBP-1,5 by the electron transport chain.

In order to assess acclimation of photosynthetic capacity (maximum velocity of carboxylation and capacity of regeneration of RuBP-1,5 by the electron transport chain) to temperature and moisture, we measured responses of apparent photosynthetic capacity ($V_{C\max,\mathrm{Ci}}$ and $J_{\max,\mathrm{Ci}}$ and their ratio) to a full factorial design of four tree species under experimental warming and rainfall reduction treatments. Contrasting ambient temperature vs +3.2 °C warming treatments were implemented throughout the 2015 growing season, in conjunction with ambient and reduced rainfall treatments (see Materials and methods section) that resulted in moderately higher (75th percentile) and moderately lower (20th percentile) rainfall, respectively than the 100-year average for May–September for this site. We selected two boreal and two temperate species and measured A/C_i curves for plants from all warming × rainfall combinations under controlled environmental conditions at three different measuring temperatures (18, 25 and 32 °C), to test a series of hypotheses (see below).

This work complements and extends a related study (Stefanski et al. 2020) that measured plants in situ in the same experimental treatments; those measurements were made under field soil moisture conditions at variable temperatures that averaged near 27 °C (±2.2 SD). Measurements reported herein enable us to assess responses in different ways than in that prior paper.

Table 1. Summary of scientific papers presenting results of manipulative experiments that reported maximum carboxylation capacity of Rubisco (V_{Cmax}) and maximum capacity of regeneration of RuBP-1,5 (J_{max}) at both different growing temperatures and measurement temperatures. In this table, we describe the type of experimental design, type of plant species, length of study and the temperature differential among levels of the warming treatment (T_{growth}), as well as whether the photosynthetic parameters were significantly affected. Those that were affected and are shown without asterisk changed as hypothesized (i.e., down-regulated at higher temperatures); with asterisk means results were significant but in the opposite direction. GC-GH—growth chamber-green house style, WTC—whole-tree chamber, FG—field garden, WCC—whole crown chamber, OTC—open top chamber, OPF—open plots field. In the columns for the interaction of warming (T_{growth}) and leaf temperature ($T_{measure}$), 'ns' means no significant differences between treatments, 'Yes*' means significant differences were found between the treatments and they have a positive slope; 'Yes' means significant differences were found and warming and leaf temperature showed a negative slope.

Publication	Experiment type	Growing medium	Plant model type	Growth temperature difference	Length of the study	$T_{growth} \times T_{measure}$	
						V_{Cmax}	J_{max}
Saez et al. (2018)	GC-GH	Pots fertilized	*Deschampsia antartica* and *Colobanthus quitensis*	Min 5 °C, max 11 °C (5, 10 and 16 °C)	1 season	Yes*	–
Lamba et al. (2018)	WTC	Field experiment	*Picea abies* (Norway spruce)	3 °C	3 years	ns	–
Smith and Dukes (2017)	GC-GH	Pots fertilized	22 species (wide range of growth form and habitat of origin)	Min 5 °C, max 20 °C (15, 20, 25, 30, 35 °C)	Variable (but within a season)	Yes*	Yes*
Crous et al. (2013)	WTC	Pots fertilized	*Eucaliptus globulus*	3 °C (amb. +3 °C)	2 seasons	Yes (*) (season dependent)	Yes (*) (season dependent)
Ge et al. (2012)	GC-GH	Microcosm fertilized	*Phalaris arundinacea* (boreal grass)	3.5 °C	2 seasons	Yes*	Yes*
Dillaway and Kruger (2010)	FG	Field fertilized	Four tree species	Variable (latitudinal differences)	1 season	ns	ns
Silim et al. (2010)	GC-GH	Pots fertilized	*Populus balsamifera*	8 °C	1 season	ns	Yes
Kositsup et al. (2009)	GC-GH	Pots fertilized	*Hevea brasiliensis* (rubber tree)	10 °C	1 season	Yes*	Yes*
Shen et al. (2009)	OTC	Field experiment	*Gentiana staminea*	1.6 °C avg. (max 7.3 °C)	1 season (but long-term experiment)	ns	Yes
Niu et al. (2008)	OPF	PVC tubes	Four C_3 grasses and forbes and two C_4 grasses	–	1 season	Yes* (but sp-specific)	Yes* (but sp-specific)
Warren (2008)	GC-GH	Pots fertilized	*Eucaliptus regnans*	15 °C	1 season	Yes	ns
Way and Sage (2008)	GC-GH	Pots fertilized	*Picea mariana*	8 °C	1 season	Yes	ns
Ishikawa et al. (2007)	GC-GH	Pots fertilized	*Plantago asiatica* (three ecotypes)	15 °C	1 season	Yes*	Yes
Yamori et al. (2006)	GC-GH	Pots fertilized	*Spinacia oleracea*	15 °C	1 season	Yes*	–
Yamori et al. (2005)	GC-GH	Pots fertilized	*Spinacia oleacea*	15 °C	1 season	Yes*	Yes
Bernacchi et al. (2003)	GC-GH	Pots fertilized	*Nicotiana tabacum*	10 °C	1 season	–	Yes (*)
Bunce et al. (2000)	GC-GH	Pots fertilized	Eight herbaceous species	10 °C	1 season	Yes* (but sp-specific)	–
Hikosaka et al. (1999)	GC-GH	Pots fertilized	*Quercus myrsinaefolia*	15 °C	1 season	Yes*	Yes*
Wang et al. (1996)	OTC	Field experiment	*Pinus sylvestris*	Variable (2–20 °C)	1 season (3-year experiment)	Yes*	Yes*

First, by measuring well-hydrated leaves in laboratory conditions under controlled temperatures, we could assess the Type II acclimation of photosynthetic capacity under standardized measurement temperature and moisture conditions (Type II of Smith and Dukes 2013). This provided a useful complement to the prior in situ measurements (Stefanski et al. 2020) that assessed acclimation under differing growth conditions rather than under standardized ones. Second, by measuring A/C_i curves at three different measurement temperatures we are able to assess Type I acclimation, which was not part of the prior field measurement report (Stefanski et al. 2020).

Hypotheses

H1: Type I acclimation of short-term thermal sensitivity of apparent photosynthetic capacity ($V_{C\mathrm{max,Ci}}$, $J_{\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$:$V_{C\mathrm{max,Ci}}$) We hypothesize that as a result of basic biochemistry all plants will increase $V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ under higher rather than lower measurement temperatures (H1.1; Hikosaka et al. 1999, 2006, Dreyer et al. 2001, Medlyn et al. 2002, Way and Yamori 2014). However, because the boreal species may have already maximized their potential acclimation under conditions prevailing near their warm temperature limit, we hypothesize that their increases from 18 to 25 to 32 °C will be smaller (H1.2). We also hypothesize (H1.3) that plants grown at elevated temperatures and at higher moisture availability would develop greater overall biochemical capacity and thus show a greater ability to increase photosynthetic capacity with increasing short-term temperatures (Type I acclimation). Finally, we hypothesize (H1.4) that the modified Arrhenius function, a widely used equation for adjusting $V_{C\mathrm{max}}$ and J_{max} to 25 °C when measured at other temperatures, will allow us to predict our 25 °C measurements from those made at 18 and 32 °C under all the combinations of warming and rainfall reduced conditions of the study.

H2: Type II acclimation of photosynthetic parameters to warming We hypothesize that temperate and boreal species have been selected to maintain a degree of homeostasis of their realized photosynthetic performance ($V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ under treatment conditions) in the face of the considerable temperature variation they experience. In order to have similar $V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ when grown and measured at higher temperatures, they must down-regulate $V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ (as assessed when measured at common temperature, e.g., $V_{C\mathrm{max,Ci}}$ at 25 °C and $J_{\mathrm{max,Ci}}$ at 25 °C) when growing at higher temperatures (H2.1), a Type II acclimation.

Additionally, the boreal species might respond differently to warming than the temperate species, as they live near their warm-range margins. Current understanding is insufficient to pose a well-supported predictive hypothesis about such species differences, but it is possible that boreal species have less capacity to adjust to higher temperatures than temperate species and will show smaller adjustments of $V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ to long-term warming (H2.2).

H3: Type II acclimation of photosynthetic parameters to reduced rainfall Drier conditions usually cause stomatal closure, including for these species (Reich et al. 2018). As a consequence, C_i will decrease, reducing photosynthetic rates experienced in the field. We hypothesize that in response to increased time spent at low stomatal conductance, plants will down-regulate the $V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ to trim excessive and costly photosynthetic capacity (H3.1). Further, we would expect a species-specific down-regulation response, varying with the species drought tolerance (H3.2).

H4: Type II acclimation to combined effects of warming and rainfall reduction We expect that the combined exposure to warming and rainfall reduction will have an additive effect on $V_{C\mathrm{max,Ci}}$ and $J_{\mathrm{max,Ci}}$ (H4.1); i.e., arithmetically equivalent to the sum of the individual effects of warming and rainfall reduction when they are applied independently. This is a highly uncertain prediction as the exact mechanisms of impact of the two environmental drivers are poorly understood. Moreover, we expect the $J_{\mathrm{max,Ci}}$:$V_{C\mathrm{max,Ci}}$ ratio will remain relatively constant due to a simultaneous down-regulation of the less limiting one of them (H4.2). For example, in the case of a low diffusion of CO_2 due to stomatal closure that would reduce the $V_{C\mathrm{max,Ci}}$ we would expect a simultaneous down-regulation in $J_{\mathrm{max,Ci}}$ in order to save resources and maximize the energetic economy of the plant.

Materials and methods

Location and species

In the present work, we report on measurements made in 2015 of saplings of four widely distributed North American tree species of ecological and economic interest in boreal and temperate forests. They were planted in the long-term chamber-free climate change experiment 'Boreal Forest Warming at an Ecotone in Danger' (B4WarmED) (Reich et al. 2015, Rich et al. 2015, Sendall et al. 2015) at a field station of the University of Minnesota, the Cloquet Forestry Center (46°40′46″N, 92°31′12″W, 382 m above sea level, near Cloquet, MN, USA). The four species were: *Betula papyrifera* Marsh., a boreal angiosperm species, *Pinus banksiana* Lamb., a boreal gymnosperm, *Acer rubrum* L., a temperate angiosperm species and *Pinus strobus* L., a temperate gymnosperm (Reich et al. 2015). The plants were 1–2 years old when they were planted and they were growing for three to four seasons under the treatments when they were measured (see Table S2 available as Supplementary

Data at *Tree Physiology* Online, for the sizes of the different species).

The overall experimental design was a two long-term warming, two summer rainfall reduction, four species full factorial, with warming and rainfall treatments at plot scale and all species included in all plots (see Rich et al. 2015 for more details). We used a total of 12 circular plots 3 m in diameter, with three unique replicates per plot-level treatment. The warming treatment included two levels of simultaneous above and belowground warming (ambient and +3.2 °C above ambient temperature), implemented using infrared lamp heaters for aboveground and soil heating cables for belowground, with dummy lamps and cables in the ambient plots (Rich et al. 2015). Warming was implemented from early spring to late fall each year, via a feedback control that acts concurrently and independently at the plot scale to maintain a fixed temperature differential from ambient conditions above- and below ground. On average, we achieved 24 h day^{-1} and daytime (9:00–15:00 solar time) warming of +3.2 °C (1 June 1–30 September) in 2015 (Stefanski et al. 2020).

Rainfall treatment included two levels; ambient and 40% of precipitation removed from 1 June to 30 September. See Figure S2c and d available as Supplementary Data at *Tree Physiology* Online to examine the pattern of rainfall removed and the impact of the treatment on volumetric water content (VWC). This treatment was achieved by installing manually operated rainout shelters (Stefanski et al. 2020) composed of an overhead structure of 4.5 × 4.5 m roof tarp attached at 4 m above the soil. This tarp was tilted towards southwestern corner, where rain collection barrel with overflow distribution hose was located. All rainout removal plot tarps were manually operated and were unfurled shortly before an upcoming rain event, if the cumulative captured rainfall for the season was below the 40% threshold of total summer rainfall, and furled back shortly after rain. The tarps were kept furled when the current rainfall removal was higher than the target of 40% (see Figure S2c, available as Supplementary Data at *Tree Physiology* Online, shows the pattern of precipitation by day).

Rainfall from 1 May to 30 September in 2015 in ambient and reduced rainfall treatments represents the 75th and 20th percentiles when compared with ambient rainfall in Cloquet for those months over a 100-year period; hence, the two treatments represent an intermediately rainy (in the mid-range of those wetter than average) growing season and an intermediately dry growing season (i.e., in the mid-range of those drier than average). Moreover, VWC on average for days when measurements were made were similar to the average for all of May–September, so sampled days reflect well the mean VWC as influenced by rainfall patterns. To assess the likely impacts of rain treatments on extent of 'stress,' we used impacts on in situ leaf diffusive conductance as a guide. Using the linear relationship of leaf stomatal conductance (g_s) vs VWC for these four species from 2009 to 2011 (Reich et al. 2018), we made an estimate of likely impact of treatments on g_s via influence on VWC. Assuming these relationships held in 2015, mean VWC in low rain, ambient temperature would have resulted in mean g_s of 5–8% lower (range among the four species) than expected due to mean VWC in ambient rain, ambient temperature. Moreover, VWC in low rain and elevated temperature treatment would have resulted in mean g_s of 16–23% lower (again, range among species) than g_s expected based on VWC in the ambient rain and elevated temperature treatment. These calculations suggest that the low rainfall treatment influenced plants through diminished mean VWC, but that they likely maintained leaf diffusive conductance at 80–90% as high as in ambient rainfall plants (in their respective temperature treatments). Thus, the rainfall reduced treatment resulted in a mild water stress for the plants as evidenced by the small reductions in stomatal conductance observed across the treatments (a half or a third of those of ambient plants; see Table S4 and Figure S3 available as Supplementary Data at *Tree Physiology* Online).

A/C_i curve measurements

Full expanded and healthy leaves of the selected species were collected every morning at each measuring date. Petioles of single leaves in the case of angiosperm species or the apical 10-cm branch, in the case of gymnosperms, were cut underwater and maintained submerged in floral water pick tubes. The detached leaf samples were stored in dark conditions and transported immediately in a portable cooler to the laboratory. Before measurement, the samples were placed in a growth chamber and left to adjust to the measuring conditions of light and temperature for 30 min. The curves were performed using Portable Photosynthesis Systems (Li-6400XT, Li-Cor, Lincoln, NE, USA) that applied a sequence of 10 measurements defined by their concentration of CO_2 (400, 325, 250, 125, 50, 400, 650, 900, 1200, 1500 p.p.m. of CO_2), a saturating light intensity of 1200 μmol photons m^{-2} s^{-1} and an air flow of 500 μmol s^{-1}. For each sample, the A/C_i curve procedure was conducted at three different measuring temperatures (18, 25 and 32 °C). Measurement temperatures were achieved using growth chambers in which the plant sample and the leaf chamber of the portable photosynthesis system was placed. Previous tests made in another gas exchange experiment in the same project (acclimation of A_{net} to warming; Sendall et al. 2015) showed a significant strong correlation (close to 1:1) between the stomatal conductance and photosynthesis values of leaves attached and detached from the plant measured in equivalent conditions.

The measurements were conducted twice per species per plot during the summer of 2015 yielding 148 samples (over 400 individual curves) from July to August of 2015. The measured values of photosynthetic assimilation were adjusted by the projected area of the needles placed into the chamber (or leaves in the case the broadleaves that did not fully fill the area of the chamber). Projected area was determined using a flatbed scanner, after which the resulting images were analyzed with the ImageJ software (Schneider et al. 2012). The data were corrected for gasket diffusion (Bruhn et al. 2002, Manual Li-6400XT 2009) and then screened for outliers. First, we extracted the residuals from the linear regressions of log-transformed C_i and C_a and of modeled A versus measured A. Modeled A was fitted applying the 'fitaci' function from the 'plantecophys' (Duursma 2015) R package, which implements the FvCB model equations. From modeled A and measured A data, we calculated the interquartile ranges (the difference between 25th and 75th percentiles). Then, we extended beyond the borders of those interquartile ranges in both directions by twice their values. All points outside of those intervals were removed from the dataset. The remaining A/C_i data points were used to fit the FvCB model and calculate $V_{C\max,\mathrm{Ci}}$ and $J_{\max,\mathrm{Ci}}$ applying the 'fitaci' function from the 'plantecophys' (Duursma 2015) R package.

The conversion of values of photosynthetic capacity from their measuring temperature to the standard 25 °C was conducted directly by 'plantecophys' package internal functions using the peaked Arrhenius equation (modified Arrhenius function; Johnson et al. 1942):

$$k_\mathrm{T} = k_{25}\,\mathbf{exp}\left[H_\mathrm{a}\left(T_l - T_\mathrm{ref}\right) / \left(T_\mathrm{ref} R T_l\right)\right] \frac{1 + \mathbf{exp}\left(\frac{T_\mathrm{ref}\Delta S - H_\mathrm{d}}{T_\mathrm{ref} R}\right)}{1 + \mathbf{exp}\left(\frac{T_l \Delta S - H_\mathrm{d}}{T_l R}\right)} \tag{1}$$

k_T and k_{25} are the values of the photosynthetic capacity ($V_{C\max}$ or $J_{\max}$) at the measuring temperature and at reference temperature (25 °C) respectively. H_a and H_d are the activation and deactivation energies respectively. ΔS is the value of the entropy, T_l and T_ref are the leaf temperatures (in Kelvin), during the measurement and as reference (298 K) and R is the universal gas constant (8.314 J mol^{-1} K^{-1}).

All the calculations in this section were conducted in R version 3.5.2 (http://www.r-project.org/).

Photosynthetic capacity calculation from A/C$_i$ curves

In this study, we are calculating apparent $V_{C\max}$ and $J_{\max}$ ($V_{C\max,\mathrm{Ci}}$, $J_{\max,\mathrm{Ci}}$), assuming an infinite conductance in sub-stomatal cavities (g_m); i.e., we use intercellular CO_2 concentration at the mesophyll (C_i) instead the concentration of CO_2 at the chloroplast (C_c), where the process of carboxylation occur. Because of that potential acclimation, especially related to rapid changes in water availability, could remain hidden.

$V_{C\max,\mathrm{Ci}}$, $J_{\max,\mathrm{Ci}}$ and their ratio were determined from formulas (Farquhar et al. 1980, Medlyn et al. 2002, Dubois et al. 2007) based on the mechanistic model described by Farquhar et al. (1980).

$$A_\mathrm{c} = \frac{V_{C\mathbf{max}}\left(C_\mathrm{i} - \Gamma^*\right)}{C_\mathrm{i} + K_\mathrm{c}\left(1 + \frac{O}{K_\mathrm{o}}\right)} - R_\mathrm{d} \tag{2}$$

where $V_{C\max}$ is the apparent maximum rate of Rubisco carboxylation, C_i is the intercellular CO_2 partial pressure, Γ^* is the photosynthetic compensation point, K_c and K_o are the Michaelis–Menten constants of Rubisco for CO_2 and O_2, respectively, O is the partial pressure of O_2 and R_d the non-photorespiratory CO_2 evolution; and

$$A_\mathrm{j} = \frac{J\left(C_\mathrm{i} - \Gamma^*\right)}{4C_\mathrm{i} + 8\Gamma^*} - R_\mathrm{d} \tag{3}$$

where J is the rate of electron transport, C_i is the intercellular CO_2 partial pressure, Γ^* is the photosynthetic compensation point and R_d is the non-photorespiratory CO_2 evolution.

For the temperature dependency of Γ^*, K_c and K_o were used the method described in Medlyn et al. (2002), which in turn were based in the values originally described in Bernacchi et al. (2001).

There is a third mechanism described in the literature that can limit the carbon assimilation. It is based in the triose phosphate assimilation (TPU) but it is considered significant mainly at low temperatures and high CO_2 concentrations (Bernacchi et al. 2009, Kumarathunge et al. 2019*a*, 2019*b*). Some authors, as Busch and Sage (2017) found it could be significant at temperatures as high as 22 °C, however, because our plants are adapted to a cold climate and TPU limitations mostly occurs at high CO_2 concentrations, unlikely to be reached in natural conditions (Bernacchi et al. 2009, Kumarathunge et al. 2019*a*, 2019*b*), we are not considering TPU limitations in our study.

The values of net carbon assimilation (A_net), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were also extracted from the data collected for each curve. For this, we used the values closest to the current concentration in the atmosphere (i.e., C_a = 400 p.p.m. CO_2).

Statistical analysis

The apparent photosynthetic capacity descriptors ($V_{C\max,\mathrm{Ci}}$, $J_{\max,\mathrm{Ci}}$ and $J_{\max,\mathrm{Ci}}$:$V_{C\max,\mathrm{Ci}}$), net carbon assimilation (A_net), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were analyzed statistically by applying a standard least squares model with warming (T_growth: ambient, +3.2 °C), rainfall reduction (rainfall: ambient, −40% summer rainfall), species (species: *A. rubrum*, *P. strobus*, *P. banksiana* and *B. papyrifera*) and measuring temperature (T_measure: 18, 25 and 32 °C) as fixed factors in a full factorial model. For the post-hoc analysis we used the Tukey HSD method. The $V_{C\max,\mathrm{Ci}}$, $J_{\max,\mathrm{Ci}}$ and $J_{\max,\mathrm{Ci}}$:$V_{C\max,\mathrm{Ci}}$ variables were log-transformed to minimize heteroscedasticity of the model. All the tests were conducted using the JMP Pro 13 statistical suite (2016, SAS institute Inc., Cary, NC, USA).

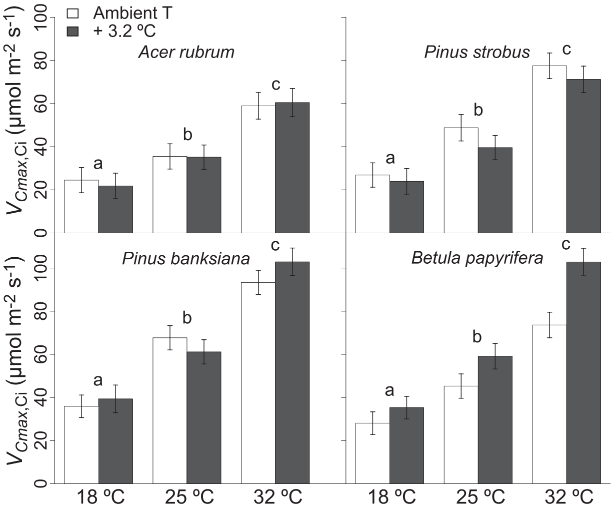

Figure 1. Effect of experimental warming (T_{growth}) on apparent maximum carboxylation capacity of Rubisco ($V_{Cmax,Ci}$) at three different measurement temperatures ($T_{measure}$, 18, 25 and 32 °C) in four tree species from different climates of origin and functional types (temperate angiosperm, *A. rubrum*; temperate gymnosperm, *P. strobus*; boreal gymnosperm, *P. banksiana* and boreal angiosperm, *B. papyrifera*). Bars represent LS (least square) means and whiskers standard errors. Empty bars represent plants growing at ambient temperatures and solid bars plants growing at +3.2 °C. Letters show statistical differences among levels of $T_{measure}$ treatment.

Results

Short-term temperature responses and Type I acclimation

All four species increased $V_{Cmax,Ci}$ and $J_{max,Ci}$ as measurement temperature increased (consistent with H1.1). Species differed significantly from one another (independent of treatments) but did not differ from one another in response to measurement temperature (inconsistent with H1.2, Table 2, Figures 1–4). Plants grown under elevated temperature or reduced rainfall did not differ from those grown in ambient conditions (inconsistent with H1.3 and H1.4 and indicative of no evidence of Type I acclimation). $V_{Cmax,Ci}$ increased on average across species by ~38.6% moving from 18 to 25 °C and by 37.9% moving from 25 to 32 °C, respectively (Figures 1 and 2). $J_{max,Ci}$ increased on average across species by 22.8% moving from 18 to 25 °C and by 19.8% moving from 25 to 32 °C (Figure 2). Because $J_{max,Ci}$ increased proportionally less than $V_{Cmax,Ci}$, the $J_{max,Ci}$:$V_{Cmax,Ci}$ ratio declined with temperature (Figure 4).

None of the species responses closely followed the predictions of a widely used equation (Eq. (1)) to convert photosynthetic parameters measured at different temperatures to a common temperature of 25 °C (Table 3). This is important to examine as such conversions are widely used in empirical and modeling papers, but rarely tested. Extrapolations from measurements made at 18 °C overpredicted $V_{Cmax,Ci}$ at 25 °C and $J_{max,Ci}$ at 25 °C by 24.4 to 50.0% and 0.2 to 25.0%

Table 2. Results for the standard sum of squares model for apparent $V_{Cmax,Ci}$, $J_{max,Ci}$ and $J_{max,Ci}$:$V_{Cmax,Ci}$ [log transformed] for the full factorial interactions of warming (ambient temperature and +3.2 °C above ambient temperature), rainfall reduction (ambient rainfall and 40% reduction of summer precipitation), $T_{measure}$ (18, 25 and 32 °C) and species (*A. rubrum*, temperate angiosperm; *P. strobus*, temperate gymnosperm; *P. banksiana*, boreal gymnosperm and *B. papyrifera*, boreal angiosperm). $P \leq 0.05$ are highlighted in bold.

Source of variance	DF	$V_{Cmax,Ci}$			$J_{max,Ci}$			$J_{max,Ci}$:$V_{Cmax,Ci}$		
		Sum of squares	*F*-ratio	*P*-value	Sum of squares	*F*-ratio	*P*-value	Sum of squares	*F*-ratio	*P*-value
Warming	1	0.25	1.64	0.20	0.01	0.10	0.75	0.38	9.21	**<0.01***
Rainfall reduction	1	0.05	0.32	0.57	0.06	0.42	0.52	0.21	5.03	**0.03***
Warming × rainfall reduction	1	0.60	3.89	**0.05***	0.54	4.20	**0.04***	$1 \cdot 10^{-3}$	0.03	0.86
Species	3	10.72	23.22	**<0.01***	14.59	37.52	**<0.01***	1.27	10.24	**<0.01***
Species × warming	3	2.26	4.89	**<0.01***	0.74	1.91	0.13	0.69	5.60	**<0.01***
Species × rainfall reduction	3	2.98	6.46	**<0.01***	2.03	5.23	**<0.01***	0.10	0.81	0.49
Species × warming × rainfall reduction	3	2.23	4.82	**<0.01***	1.73	4.45	**<0.01***	0.13	1.03	0.38
$T_{measure}$	2	43.92	142.71	**<0.01***	10.90	42.08	**<0.01***	11.09	134.60	**<0.01***
$T_{measure}$ × warming	2	0.27	0.88	0.42	0.16	0.61	0.55	0.05	0.60	0.55
$T_{measure}$ × rainfall reduction	2	0.04	0.12	0.88	0.04	0.15	0.86	$6 \cdot 10^{-4}$	$7 \cdot 10^{-3}$	0.99
$T_{measure}$ × warming × rainfall reduction	2	0.42	1.36	0.26	0.66	2.56	0.08	0.06	0.67	0.52
$T_{measure}$ × species	6	0.34	0.36	0.90	0.69	0.89	0.50	0.49	1.81	0.10
$T_{measure}$ × species × warming	6	0.24	0.26	0.95	0.31	0.40	0.88	0.11	0.46	0.84
$T_{measure}$ × species × Rainfall reduction	6	0.69	0.74	0.62	0.73	0.94	0.47	0.20	0.81	0.56
$T_{measure}$ × species × warming × rainfall reduction	6	0.37	0.40	0.88	0.28	0.36	0.90	0.08	0.33	0.92

*Asterisk indicates significant differences

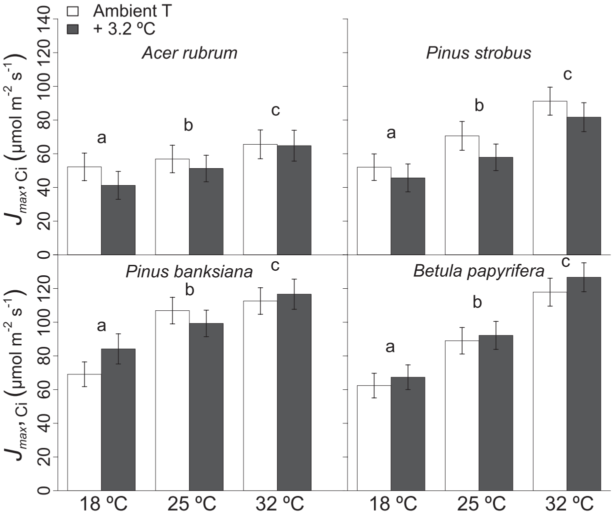

Figure 2. Effect of experimental warming (T_{growth}) on apparent maximum capacity of regeneration of RuBP-1,5 ($J_{max,Ci}$) at three different measurement temperatures ($T_{measure}$, 18, 25 and 32 °C) in four tree species. Bars represent LS (least square) means and whiskers standard errors. Empty bars represent plants growing at ambient temperatures and solid bars plants growing at +3.2 °C. Letters show statistical differences among levels of $T_{measure}$ treatment.

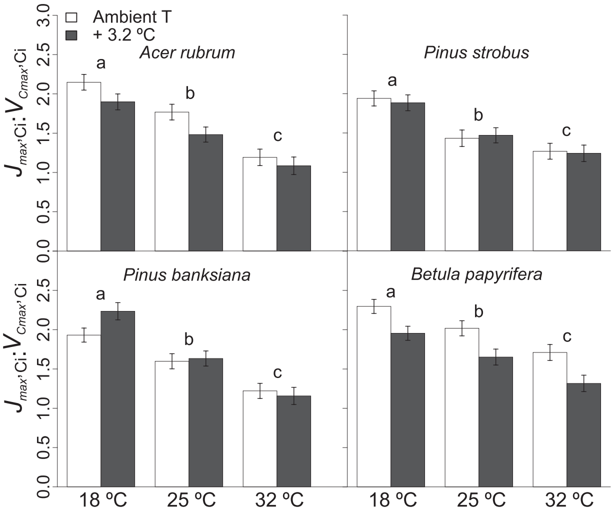

Figure 3. Effect of experimental warming (T_{growth}) on the ratio $J_{max,Ci}$:$V_{Cmax,Ci}$ at three different instantaneous temperatures ($T_{measure}$, 18, 25 and 32 °C) in four tree species. Bars represent LS (least square) means and whiskers standard errors. Empty bars represent plants growing at ambient temperatures and solid bars plants growing at +3.2 °C. Letters show statistical differences among levels of $T_{measure}$ treatment.

respectively, among the four species, whereas extrapolations from measurements made at 32 °C underpredicted $V_{Cmax,Ci}$ at 25 °C by 18.4 to 28.0% and came closer to predicting $J_{max,Ci}$ at 25 °C (response varied from −11.3% to +6.9% of values measured at 25 °C). In essence, measured responses across the 18 to 32 °C gradient were shallower than the default model, especially for $V_{Cmax,Ci}$.

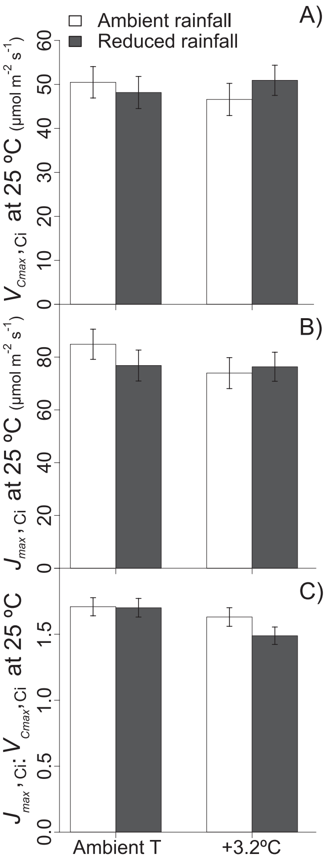

Figure 4. Interactive effect of rainfall reduction and experimental warming (T_{growth}) in (A) apparent maximum carboxylation capacity of Rubisco ($V_{Cmax,Ci}$) at 25 °C and (B) apparent maximum capacity of regeneration of RuBP-1,5 ($J_{max,Ci}$) at 25 °C. Bars represent LS (least square) means and whiskers standard errors. Empty bars represent plants growing at ambient rainfall regime and solid bars plants growing with a 40% summer rainfall reduction. Bars on the left side of the figure represent plants growing at ambient temperature and on the right side of the figure plants growing at +3.2 °C.

Type II acclimation to warming or/and rainfall reduction treatments

The similar short-term responses to temperature among species, warming treatments and rainfall treatments (i.e., no significant interactions) simplifies tests of Type II acclimation, as differences at the three measurement temperatures should align; and main effects in the overall model are diagnostic given lack of interactions. Neither warming nor rainfall reduction had a significant main effect on $V_{Cmax,Ci}$ or $J_{max,Ci}$ (Table 2), offering no support for hypotheses H2.1 and H3.1 and no evidence of a consistent Type II acclimation; in essence, when measured at a standardized temperature these parameters did not differ consistently among treatments. However, we detected significant interactions between warming and rainfall reduction ($P < 0.05$) for both metrics (Table 2); these were driven by inconsistent responses among species (Figure 5). For example, species differed significantly in their response to rainfall reduction and the

Table 3. Values of apparent $V_{Cmax,Ci}$ at 25 °C and $J_{max,Ci}$ at 25 °C (± SE) for four tree species averaged across warming and rainfall reduced treatments. Central columns show the values for both variables measured at 25 °C. The other columns show the values for the same variables converted to 25 °C from values measured at 18 and 32 °C using the functions available in the R package 'plantecophys' (see Materials and methods section for references) that uses the modified Arrhenius equation (Eq. (1)).

Species	$V_{Cmax,Ci}$ at 25 °C (μmol m^{-2} s^{-1})			$J_{max,Ci}$ at 25 °C (μmol m^{-2} s^{-1})		
	From 18 °C	25 °C (measured)	From 32 °C	From 18 °C	25 °C (measured)	From 32 °C
Acer rubrum	53.1 ± 3.2	35.4 ± 3.1	28.1 ± 2.5	67.6 ± 4.4	54.1 ± 3.5	52.5 ± 4.1
Pinus strobus	56.9 ± 3.4	43.5 ± 2.9	35.5 ± 3.2	70.3 ± 4.5	63.1 ± 4.5	69.3 ± 4.9
Pinus banksiana	80.1 ± 7.0	64.4 ± 4.2	46.4 ± 2.9	106.4 ± 10.2	103.3 ± 7.5	91.6 ± 5.9
Betula papyrifera	67.2 ± 3.8	52.6 ± 4.1	41.1 ± 4.1	91.2 ± 4.7	91 ± 6.1	95.5 ± 8.2

interaction of rainfall × warming (species × warming × rainfall interaction, $P < 0.01$, Table 2). *Acer rubrum* decreased $V_{Cmax,Ci}$ and $J_{max,Ci}$ under low rainfall treatment, whereas the other three species increased $V_{Cmax,Ci}$ and $J_{max,Ci}$ (Table 2, Figure 5). The four species responded differently to the combination of warming × rainfall reduction (Figure 5, Table 2).

In the case of the ratio of $J_{max,Ci}$:$V_{Cmax,Ci}$, we found significant effects of both treatments as main factors and significant differences among species in their response to warming (Table 2, Figure 6). Plants growing in warmer or drier conditions showed a significant down-regulation of this index. However, the down-regulation of $J_{max,Ci}$:$V_{Cmax,Ci}$ with experimental warming was observed in two of the four species with the other two having no response (Figure 6, Table 2). Finally, species differed on average in $J_{max,Ci}$:$V_{Cmax,Ci}$, with *B. papyrifera* having higher values than all the other three species.

Net carbon assimilation (A_{net}), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i)

Net photosynthetic assimilation and stomatal conductance differed among measurement temperatures and species (see Tables S3 and S4 available as Supplementary Data at *Tree Physiology* Online). The other main factors related to the climate, warming and rainfall reduction did not show a significant effect, however, they had an interactive effect between species and rainfall reduction for stomatal conductance and among species, rainfall reduction and warming in net carbon assimilation (see Tables S3 and S4 available as Supplementary Data at *Tree Physiology* Online).

Discussion

Most of the current knowledge about the effects of climate warming and water availability on photosynthesis capacity are based on studies made in conditions both more stable (i.e., mostly laboratory studies; see Table 1 and Table S1 available as Supplementary Data at *Tree Physiology* Online for more examples of temperature papers) and more strongly contrasting (i.e., larger differences among treatments; see Table 1 and Table S1 available as Supplementary Data at *Tree Physiology* Online, for more examples of temperature papers) than the expected climate change of this century. Moreover, in the case of papers related to photosynthetic capacity and water availability in trees, there is a skewed focus on Mediterranean and semi-arid climates, where highly specialized and adapted species grow (Xu and Baldocchi 2003, Martin-StPaul et al. 2012, Cano et al. 2013, Haworth et al. 2018).

Those studies are valuable to understanding the potential mechanisms plants may make in response to these drivers, but may not be as useful in detecting the magnitude of such responses under projected global change. Our study begins to fill that gap by assessing whether plants in ecologically realistic contrasting thermal growing conditions modify their photosynthetic biochemistry and its sensitivity to short-term temperatures. Our results suggest (i) that boreal trees possess considerable short-term plasticity to adjust their photosynthetic biochemistry to altered temperatures, which may allow homeostasis in the long-term temperature-response relation of $V_{Cmax,Ci}$ and $J_{max,Ci}$, (ii) that the quantitative shape of short-term sensitivity was not well predicted by current theory and (iii) that expectations of considerable sensitivity of photosynthetic parameters to modest long-term temperature change, based on prior studies under controlled and markedly contrasting temperature treatments, should be revisited. In this discussion, we will explore possible reasons for these results.

As expected, short-term variation in leaf temperature ($T_{measure}$) had a strong impact on photosynthetic capacity (Way and Sage 2008, Silim et al. 2010, Urban et al. 2017). However, the response to short-term temperature variation was unaffected by other variables such as prior growth temperature or drought (water availability), i.e., a lack of evidence of Type I acclimation. That is contrary to what we expected in our hypotheses and findings of some studies (Wang et al. 1996, Smith and Dukes 2017, Urban et al. 2017), but compatible with the results of other authors (Dillaway and Kruger 2010, Lamba et al. 2018). Moreover, species experiencing long-term temperature and rainfall treatments did not on average consistently acclimate (down-regulate) photosynthetic capacity

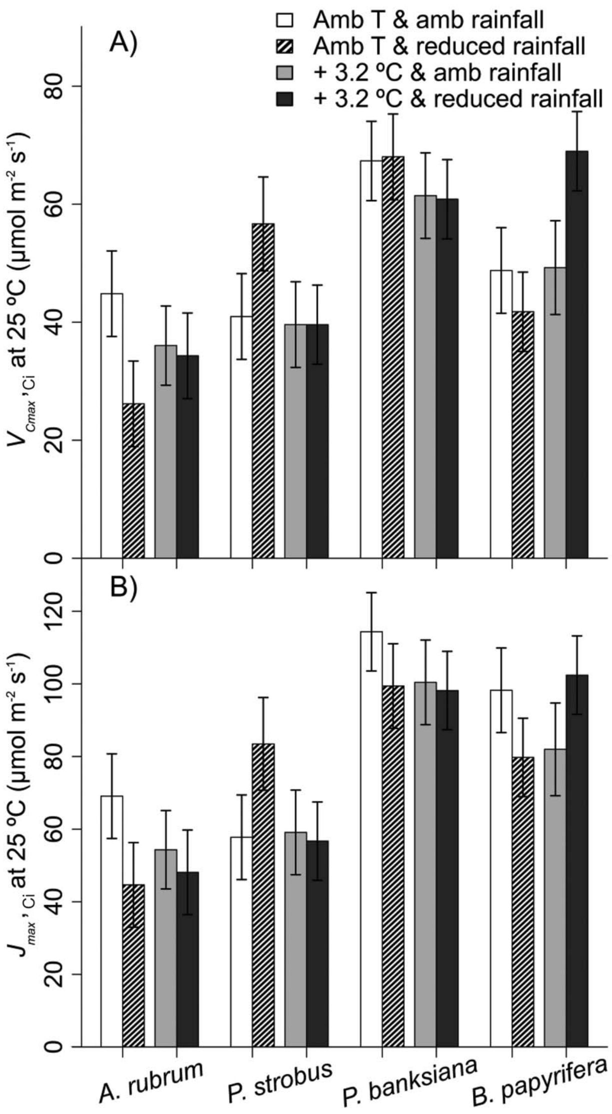

Figure 5. Interactive effect of rainfall reduction and experimental warming (T_{growth}) in four tree species arranged from left to right in the figure by the position of the center of their distribution range (from south to north). The different panels represent: (A) apparent maximum carboxylation capacity of Rubisco ($V_{Cmax,Ci}$) measured at 25 °C, (B) apparent maximum regeneration capacity of RuBP-1,5 by the electron transport chain ($J_{max,Ci}$) measured at 25 °C. Bars represent LS (least square) means and whiskers standard errors.

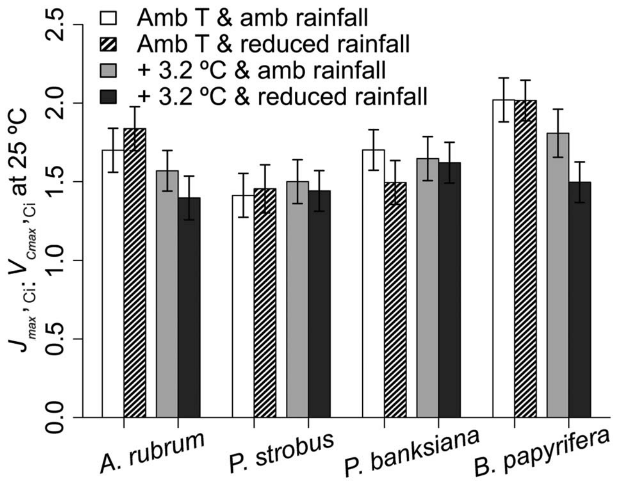

Figure 6. Interactive effect of rainfall reduction and experimental warming (T_{growth}) in four tree species arranged from left to right in the figure by the position of the center of their distribution range (from south to north). Bars represent LS (least square) means for the ratio $J_{max,Ci}$:$V_{Cmax,Ci}$, from $V_{Cmax,Ci}$ and $J_{max,Ci}$ measured at 25 °C, and whiskers standard errors.

descriptors when compared at standardized temperatures; i.e., there was a lack of evidence for a general tendency to exhibit Type II acclimation. These results suggest that many tree species, such as two of those in this study, that are adapted to strongly seasonal and varying climates rely on short-term plasticity to adjust photosynthetic parameters, and at least across the range of temperatures and moisture availability experienced in this study, may be relatively invariant in terms of their photosynthetic parameters. Others might up-regulate or down-regulate photosynthetic capacity, as noted for one species each herein.

We also hypothesized that species would differ in responses to short-term temperature variation. However, contrary to our expectations (H1.2), boreal species did not show smaller increases of $V_{Cmax,Ci}$ or $J_{max,Ci}$ than temperate species as short-term leaf temperature increased (and all species showed similar responses).

Although we found no significant acclimation of apparent photosynthetic capacity to long-term warming nor any effect of growth temperature (warming) on how apparent photosynthetic capacity responded to the short-term variation in leaf temperature ($T_{measure}$), some prior work has found significant effects of that kind (e.g., Wang et al. 1996, Bauerle et al. 2007, Way and Sage 2008, Kositsup et al. 2009, Crous et al. 2013). Why did we find different results? The answer may involve specifics of our study and prior ones.

First, the results of prior studies were not uniform, with responses varying depending on the species used and the conditions of the experiment (e.g., Hikosaka et al. 1999, Bernacchi et al. 2003, Niu et al. 2008). For example, Hikosaka et al. (1999) found that in *Quercus myrsinaefolia*, V_{Cmax} and J_{max} in plants growing at 30 °C were more sensitive (steeper relative response) to short-term changes in leaf temperature ($T_{measure}$) than plants growing at 15 °C. However, Smith and Dukes (2017) for perennial C_3 species and Bernacchi et al. (2003) for *Nicotiana* found the opposite trend with higher sensitivity at colder grown temperature, and Warren (2008) in *Eucalyptus regnans* did not find any interactive response between the growing temperature and the leaf temperature ($T_{measure}$), Other short-term studies, including those with cold-climate gymnosperms, observed no difference in short-term sensitivity to temperature of plants grown at contrasting temperatures (Way and Sage 2008, Lamba et al. 2018), similar to our findings.

Second, we posit that differences in experimental design among many prior experiments described above and our experiment may have contributed to the different results found in some other experiments from those in ours. For example, in many studies, plants were grown in pots in growth chambers; moreover, they were usually grown and/or compared (often both) across much larger contrasting growth temperatures than in our study, and with fixed temperatures throughout the day (day-night or single fixed temperatures; Hikosaka et al. 1999, Warren 2008, Way and Sage 2008, Kositsup et al. 2009, Silim et al. 2010, Crous et al. 2013, Perdomo et al. 2016, Smith and Dukes 2017). Thus, when photosynthesizing, plants in contrasting temperatures in those studies were always subject to large temperature differences across treatments and little to no temporal variation within treatments. In contrast, for our studied plants, mean temperature differences among warming treatments (3.2 °C) were modest compared with the 10–15 °C differences among treatments in prior laboratory studies (Hikosaka et al. 1999, Warren 2008, Kositsup et al. 2009, Perdomo et al. 2016), and were smaller than the within-treatment seasonal variation among mean daily daytime temperatures or the mean intra-day temperature range during well-lit daytime hours (roughly 8 °C). Plants might adjust photosynthetic biochemistry differently when exposed to modest mean differences in regimes characterized by high and unpredictable temporal variation (hours, days, weeks) versus large mean differences among regimes characterized by negligible temporal variation. The latter offers a much easier cue for physiological acclimation than the former, and perhaps also a much greater potential advantage of any such variation. Consistent with these ideas, other studies that applied a similar amplitude of growth temperatures as we applied also did not find evidence of acclimation to warming (Wang et al. 1996, Lamba et al. 2018).

Our results also found no evidence for down-regulation of $V_{\mathrm{Cmax,Ci}}$ and $J_{\mathrm{max,Ci}}$ with rainfall reduction (refuting H3.1). One might argue that perhaps part of the reason was that during the entire measuring process the leaves were maintained in optimal conditions, at 25 °C and well hydrated, with the petioles inserted in floral water pick tubes submerged in water. However, our test was intended to examine plants grown under contrasting conditions but measured under standardized temperature and water availability, so this context is required for the particular kind of acclimation we were testing. Moreover, for the same species and experimental treatments but measured in intact leaves in the field, Stefanski et al. (2020) also found no significant differences in $V_{\mathrm{Cmax,Ci}}$ at 25 °C or $J_{\mathrm{max,Ci}}$ at 25 °C between plants growing in ambient versus reduced rainfall conditions. As soil moisture was not consistently very low during the time periods of either study, it is possible that water deficits induced by reduced summer precipitation may have simply been not intense enough to have had any effect of the biochemistry of photosynthesis. This interpretation is consistent with conclusions that the main direct way drought affects photosynthesis capacity (V_{Cmax} and J_{max}) is by modifying stomatal and mesophyll conductances, changing the patterns of gas diffusion between the atmosphere and the chloroplast (Flexas et al. 2004). Flexas et al. (2002) ascribed typical limitation of photosynthesis in response to mild and moderate water stress to stomatal closure and demonstrated that an increase of biochemical limitation of photosynthesis only occurs as water stress becomes more severe (Flexas et al. 2002, 2004, Flexas and Medrano 2002, Cano et al. 2014), typically when stomatal conductance drops below the 0.05 mol H_2O m^{-2} s^{-1} (Flexas et al. 2002). This level was rarely observed in our data set (see Figure S3a–d available as Supplementary Data at *Tree Physiology* Online), and it appears that only mild to moderate water stress was experienced by seedlings in our study.

Our results support the hypothesis of down-regulation of the ratio $J_{\mathrm{max,Ci}}$:$V_{\mathrm{Cmax,Ci}}$ with temperature elevation or rainfall reduction. Similar results, with a down-regulation of J_{max}:V_{Cmax} ratio, were found by other authors for plants growing at higher temperatures (Dreyer et al. 2001, Robakowski et al. 2002, Misson et al. 2006, Lin et al. 2013) or drought conditions (Limousin et al. 2010, Galle et al. 2011, Zhou et al. 2016). However, there were also other studies in the literature where the values of J_{max}:V_{Cmax} were up-regulated with higher levels of drought (Egea et al. 2011, Cano et al. 2013), while yet other studies found J_{max}:V_{Cmax} does not vary in response to limited water availability (Meir et al. 2007, Kelly et al. 2016) or growth temperature (Fürstenau Togashi et al. 2018). Our results add to the evidence suggesting that down-regulation of the $J_{\mathrm{max,Ci}}$:$V_{\mathrm{Cmax,Ci}}$ ratio is a common, but not universal response to warming and drought.

In comparing the outcomes of the conversion of the $V_{\mathrm{Cmax,Ci}}$ and $J_{\mathrm{max,Ci}}$ values measured at 18 and 32 to 25 °C ($V_{\mathrm{Cmax,Ci}}$ at 25 °C and $J_{\mathrm{max,Ci}}$ at 25 °C) by the modified Arrhenius model (Eq. (1)), we observed a systematic mismatch between the values estimated versus measured at 25 °C. The mismatch was systematically larger for $V_{\mathrm{Cmax,Ci}}$ at 25 °C than for $J_{\mathrm{max,Ci}}$ at 25 °C. $V_{\mathrm{Cmax,Ci}}$ at 25 °C was overestimated by 31% when calculated from the measurements sampled at 18 °C and underestimated by 22% when estimated from measurements at 32 °C. Scafaro et al. (2017) had similar findings with similar magnitudes of biasing for V_{Cmax}. They did not test for J_{max}. Jointly, these findings suggest that models that use the modified Arrhenius equation (Eq. (1)) method to convert V_{Cmax} and J_{max} to 25 °C should use caution as this conversion method being inaccurate in many cases (see also Kumarathunge et al. 2019*a*, 2019*b*).

In summary, there was no evidence of consistent long-term acclimation in apparent photosynthetic capacity (either $V_{\mathrm{Cmax,Ci}}$

or $J_{\mathrm{max,Ci}}$ at a standard temperature, Type II acclimation), nor was there evidence of acclimation of photosynthetic responses to short-term temperature variation (Type I acclimation) for tree seedlings of four near-boreal species that had experienced experimental climate warming and/or reduced rainfall. Given many reports of acclimation to much larger environmental treatment contrasts in much more stable controlled settings (e.g., Table 1 and Table S1 available as Supplementary Data at *Tree Physiology* Online), one might question whether such studies (when contrasted with ours) have led us to falsely expect plants in nature to acclimate photosynthetic biochemistry to relatively modest climate change. Our studies in more realistic conditions subjected to smaller treatment differences support the idea that additional ecologically realistic field experiments are needed to answer this question. Doing so should be a priority, as the suite of earth system models used to predict future carbon cycles (and hence climate), including impacts of forests, are sensitive to assumptions and algorithms about the temperature sensitivity of photosynthesis at multiple temporal scales.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of Interest

None declared.

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Authors' contributions

R.B. was responsible for overall experimental implementation, conception and design of this specific study, methodology implementation, experiment execution, data collection, data analysis/interpretation and manuscript writing/revision.

A.S. was responsible for overall experimental implementation, conception and design of this specific study, methodology implementation, experiment execution and manuscript writing/revision.

R.A.M. was responsible for overall experiment conception and implementation, conception and design of this specific study and manuscript writing/revision.

P.B.R. was responsible for overall experiment conception and implementation, conception and design of this specific study, data analysis/interpretation and manuscript writing/revision.

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