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Research paper

Limited physiological acclimation to recurrent heatwaves in two boreal tree species

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The intensity of extreme heat and drought events has drastically risen in recent decades and will likely continue throughout the century. Northern forests have already seen increases in tree mortality and a lack of new recruitment, which is partially attributed to these extreme events. Boreal species, such as paper birch (*Betula papyrifera*) and white spruce (*Picea glauca*), appear to be more sensitive to these changes than lower-latitude species. Our objectives were to investigate the effects of repeated heatwaves and drought on young paper birch and white spruce trees by examining (i) responses in leaf gas exchange and plant growth and (ii) thermal acclimation of photosynthetic and respiratory traits to compare ecophysiological responses of two co-occurring, yet functionally dissimilar species. To address these objectives, we subjected greenhouse-grown seedlings to two consecutive summers of three 8-day long, +10 °C heatwaves in elevated atmospheric CO₂ conditions with and without water restriction. The data show that heatwave stress reduced net photosynthesis, stomatal conductance and growth—more severely so when combined with drought. Acclimation of both photosynthesis and respiration did not occur in either species. The combination of heat and drought stress had a similar total effect on both species, but each species adjusted traits differently to the combined stress. Birch experienced greater declines in gas exchange across both years and showed moderate respiratory but not photosynthetic acclimation to heatwaves. In spruce, heatwave stress reduced the increase in basal area in both experimental years and had a minor effect on photosynthetic acclimation. The data suggest these species lack the ability to physiologically adjust to extreme heat events, which may limit their future distributions, thereby altering the composition of boreal forests.

Keywords: climate change, drought stress, elevated CO₂, photosynthetic adjustment, thermal adjustment.

Introduction

The severity of heatwave events is increasing globally due to rising atmospheric greenhouse gases and subsequent increasing air temperatures (IPCC 2014, Kala et al. 2016). Small changes in the average air temperature can come with substantial changes in the frequency of climate extremes such as heatwaves. This rise in heatwave events appears to have contributed greatly to increasing forest mortality (Allen et al. 2010, McDowell and Allen 2015, McDowell et al. 2016, Adams et al. 2017).

Extreme heat can impact growth directly by disrupting cellular processes and indirectly through the effects of rising

leaf-to-air vapor pressure deficit (VPD). During high temperature conditions, net photosynthesis (*A*) typically declines when species' thermal limits are exceeded. Reductions in *A* can be attributed to damage to photosynthetic machinery, inactivation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), limitations to ribulose 1,5-bisphosphate (RuBP) regeneration, reduced membrane stability and increased mitochondrial respiration and photorespiration (Berry and Bjorkman 1980, Yamori et al. 2014, Teskey et al. 2015, Rashid et al. 2018a). While high air temperatures can lead to physiological damage, most plants are able to tolerate high temperatures and delay cellular damage during short-term heatwaves when water is available for evaporative cooling (Hoover et al. 2017).

However, sufficient water supply may be unlikely for the duration of a prolonged heatwave, especially for shallowly rooted species (Tang et al. 2014). Furthermore, heatwaves occurring on cloudless days expedite the use of soil water. High VPD and low soil moisture can cause xylem tensions to exceed plants' embolism thresholds, leading to hydraulic failure (McDowell et al. 2008, Sevanto et al. 2014). The combination of high VPD straining the hydraulic system and reduced photosynthetic activity has been implicated as a driver of forest mortality during prolonged heatwaves (Allen et al. 2010, 2015, McDowell et al. 2016, Adams et al. 2017).

Plants' independent reactions to heat and drought can result in opposing physiological responses, which makes predicting reactions to the combined stress difficult. Specifically, stomatal conductance (g_s) and transpiration (E) are typically reduced during drought to conserve water and, in doing so, A is also reduced (Chaves 1991, McDowell et al. 2008). In contrast, during heat stress, g_s and A can become partially or fully decoupled. At moderate heat stress, g_s and E increase to allow for leaf cooling while A is generally unaffected only partially decoupling g_s and A . When the two stressors co-occur, as they often do during heatwaves, a conflict arises in stomatal response (Chaves et al. 2016). If plants respond to drought and reduce g_s , there is a risk of thermal damage to leaf function (Ruehr et al. 2015), but if they respond to heat and so g_s increases, there is a risk of hydraulic failure (Drake et al. 2018). Moreover, there is a potential during an extreme heatwave for both photosynthetic and hydraulic dysfunction if A and E become fully decoupled where high transpiration rates and near zero A rates co-occur (Ameje et al. 2012, Slot et al. 2016, Urban et al. 2017, Drake et al. 2018).

Plants can metabolically adjust to better tolerate the changes in temperature and water availability, yet their resiliency to these stressors varies considerably. Some species have demonstrated thermal photosynthetic and/or respiratory acclimation while other species have not (Way and Sage 2008, Ow et al. 2008a, 2008b, Dillaway and Kruger 2010, Sendall et al. 2014, Reich et al. 2016, O'Sullivan et al. 2017, Kurepin et al. 2018). Comprehensive thermal acclimation requires the adjustment of both A and respiration (R), but these two processes have very different sensitivities to temperature. Photosynthesis begins to decline at lower temperatures ($<40^\circ\text{C}$) than R ($>45^\circ\text{C}$; Yamori et al. 2005). Therefore, even a small temperature increase above the photosynthetic optimum can greatly alter a plant's carbon balance (Sage et al. 2008, Way and Sage 2008, Ow et al. 2010, Way and Oren 2010, Zhang et al. 2015). To avoid disrupting the carbon balance under elevated temperatures, R must be reduced proportionally to A to maintain the A/R ratio and prevent a greater proportion of daily fixed carbon from being respired (Way and Sage 2008). Therefore, the growth and survival under future climate scenarios will depend on species' abilities to thermally acclimate both processes.

The capacity of species to acclimate to warmer temperatures appears to be broadly explained by the inherent differences in growth strategies between angiosperms and conifers. By comparing responses to temperature and drought in Mediterranean angiosperms and conifers, Carnicer et al. (2013) conclude that differences in ecophysiological traits, such as growth allometry, sensitivity to competition, hydraulic safety margins, sensitivity of stomatal conductance to VPD and carbon allocation, may favor acclimation of angiosperms. Plant photosynthetic responses to current and future heatwaves remain unclear and work has just started to identify some of the species-specific responses to these events (Ameje et al. 2012, Bauweraerts et al. 2014a, Haworth et al. 2018, Fauset et al. 2019; French et al. 2019). Furthermore, to add to the uncertainty of plant behavior during heatwaves, elevated atmospheric CO_2 (eCO_2) during these events will likely further complicate plant responses in gas exchange. Despite some evidence of photosynthetic and respiratory acclimation across species to constant, mild elevated temperatures, few attempts have been made to examine thermal acclimation to heatwaves (Hoover et al. 2017, Carter and Cavaleri 2018, Rashid et al. 2018a, 2018b), and even fewer have done so under eCO_2 conditions (Way 2013, Bauweraerts et al. 2014a, Wang et al. 2016). Because these investigations are rare, our objectives are to (i) compare responses in gas exchange and (ii) examine the potential for acclimation of A and R in two boreal tree species, a conifer (*Picea glauca*; white spruce) and an angiosperm (*Betula papyrifera*; paper birch), grown under eCO_2 and repeated heatwave stress to better understand how two co-occurring species may respond to future heatwave events.

Most paper birch and white spruce habitat is expected to experience severe heatwaves and drought in the future. Yet, little work has examined their ability to acclimate to these situations (Dillaway and Kruger 2011, 2010, Reich et al. 2015, 2016, Zhang et al. 2015, Benomar et al. 2018), and to our knowledge, there have been no investigations of these species' responses to recurrent summer heatwaves and drought under eCO_2 conditions. To address our two objectives, we will test the following hypotheses. Firstly, the heatwave and drought stress will reduce growth and leaf gas exchange, but reductions will be greatest when both stressors co-occur. Secondly, the combination of heatwave and drought stress will decouple A and g_s . Thirdly, given the recent decline observed in naturally growing populations, thermal acclimation of A and R will not occur after two summers of heatwave stress. Lastly, the effects of heatwave stress will be more severe in the conifer due to the inherent differences among functional traits in angiosperms and conifers.

Methods

Plant material and growth conditions

Native 1- to 2-year-old paper birch and 3-year-old white spruce seedlings were acquired as bare root stock from the

Wisconsin Department of Natural Resources (Wilson State Nursery, Boscobel, WI) in March 2016 (see Figure S1 available as Supplementary Data at *Tree Physiology* Online). Seedlings of the same age class that were still of manageable size for a greenhouse experiment were not available. The seedlings were planted on 1 April 2016 in 7.65 l cylindrical nursery pots (birch) or 5.68 l nursery pots (spruce) using Pro-mix HP biofungicide mycorrhizae media (Premier Tech, Inc., Rivière-du-Loup, Québec, Canada) at the University of Wisconsin-Madison Greenhouse (see Figure S2 available as Supplementary Data at *Tree Physiology* Online). For 6 weeks, the plants were watered twice per week to saturation and fertilized every other week with Peter's Professional 20-10-20 (Everris International B.V., Waardenburg, the Netherlands) at 380 ppm nitrogen. The greenhouse received natural sunlight and plants experienced temperatures ranging from 18 to 27 °C during this time. The plants were then grown in ~17 m² greenhouse rooms in the UW-Madison Biotron facility for two growing seasons (June to October 2016, April to September 2017). Following the 2016 experiment, the plants were left to overwinter in cold frames insulated with straw from October 2016 to April 2017 (at ambient atmospheric CO₂ concentrations). The rooms were on a 16 h light cycle receiving ambient sunlight except during periods of low light (photosynthetic photon flux density (PPFD) <700 mol m⁻² s⁻¹) when eight 600 W high pressure sodium fixtures provided artificial light (P.L. Light Systems Inc., Beamsville, Ontario, Canada). The atmospheric CO₂ concentration in the rooms was elevated to 700 μmol CO₂ mol⁻¹ to simulate a future intermediate emission projection scenario (Representative Concentration Pathway 6.0; IPCC 2014).

We altered daily temperature and water availability to test their effects on survival and growth of the seedlings. In 2016, there were two greenhouse rooms; one received monthly heatwaves while the other served as the control non-heatwave room. In 2017, we duplicated heatwave and control temperature conditions in two additional rooms and split plants from 2016 accordingly into their respective rooms. Temperatures in the non-heatwave rooms were set to a daily maximum between 14:00 and 18:00 h and minimum between 02:00 and 06:00 h and temperatures shifted between these values in a step-wise manner (1–2 °C h⁻¹). These temperatures were based on the 30-year weekly averages from Hayward, WI, USA (46.0130° N, 91.4846° W, 1981–2010; www.ncdc.noaa.gov), a location central to the distribution of these species in northern WI. The maximum and minimum temperature regime was changed at the start of each week. Due to the limitations of the facility, a minimum temperature of ~13 °C could be reached at night, which was at most 4 °C above the average low for Hayward. All greenhouse rooms received the same temperature regime except for heatwave days, where heatwave rooms were raised by 10 °C for 8 continuous days (see Figure S3 available as Supplementary Data at *Tree Physiology* Online). Heatwaves

occurred in the middle of June (21st–28th in 2016, 18th–25th in 2017), July (19th–26th in 2016, 16th–23rd in 2017) and August (17th–24th in 2016, 13th–20th in 2017). We chose to elevate the air temperature by 10 °C to make certain the plants experienced realistic extreme heatwaves while staying within the range of non-lethal temperatures. Air temperatures and atmospheric CO₂ concentrations were internally logged every minute. In 2017, one Easylog USB data logger (Meter, Pullman, WA) was additionally placed in each room, and air temperature and relative humidity were recorded every 15 minutes. From these data, VPD was calculated (see Figure S4 available as Supplementary Data at *Tree Physiology* Online).

In all rooms, half of the plants were well watered, and the other half received less water. In 2016, the birch trees were watered daily with each reduced-watered pot having one drip line (providing ~0.65 l week⁻¹) or well-watered pot having two drip lines (providing ~1.3 l week⁻¹). A volume of 1.3 l week⁻¹ was selected because prior to the start of the study, this amount maintained moisture within the pots while not oversaturating the media. In late May 2016, the height of the birch plants was 0.89 ± 0.03 m and by early June 2017, height had increased to 1.46 ± 0.05 m. Because of the rapid increase in plant size from the start of 2016 to start of 2017, the birch plants were watered using six (well-watered; ~4.8 l week⁻¹) or three (reduced-watered; ~2.4 l week⁻¹) drip lines per pot. The spruce plants were hand-watered twice per week with 100 ml (well-watered) or 50 ml (reduced-watered) in 2016, and 300 ml (well-watered) or 150 ml (reduced-watered) in 2017. The height of the spruce plants increased from 0.38 ± 0.01 m in late May 2016 to 0.45 ± 0.01 m in early June 2017. Several spruce plants died prior to the start of the study in 2016 due to overwatering; thus, this species had to be hand-watered with lower volumes than the birch to maintain optimal growth conditions. All irrigation administered was half strength Hoagland's Solution. As an indicator of the water status at the soil–root interface, predawn water potentials (Ψ_{PD}) were measured weekly between 4:00 and 5:00 h using a Scholander-type pressure chamber (PMS Instruments, Albany, OR). On a given sampling day, plants were randomly selected then individual leaves or branchlets ($n = 5–6$ per treatment) were cut with a razor blade and immediately measured. Hereafter, the four treatment groups are referred to as: control (C; well-watered, no heatwave); drought (D; reduced-watered, no heatwave); heat (H; well-watered, heatwave) and heat + drought (HD; reduced-watered, heatwave). There were 35 replicates per species per treatment in 2016 and 12–25 in 2017. Herbivory damage and winter kill in the cold frames caused the reduction in replicates in 2017.

Gas exchange

Gas exchange was measured on fully expanded healthy birch leaves and current year spruce needles ($n = 5–6$ per treatment)

using a portable photosynthesis system (LI-6400XT, Li-Cor, Lincoln, NE). Net photosynthesis and stomatal conductance were measured under a PPFD of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ from a red–blue LED; air flow was set to $200 \mu\text{mol s}^{-1}$ and reference CO_2 concentrations were kept at $700 \mu\text{mol mol}^{-1}$. Leaf temperature and humidity were not controlled, but we attempted to keep VPD under 2.5 kPa by adding water to the CO_2 scrub when necessary during heatwaves. Gas exchange was measured between 09:00 and 13:00 h for all treatments during both years. On each sampling date, different plants were randomly selected. One fully elongated mature leaf or branchlet was measured from the same position on each plant, about one-third of the distance below the plant apex. In 2016, there were 12 sampling dates (6 during and 6 not during heatwaves) and 10 sampling dates in 2017 (5 during and 5 not during heatwaves). To express A and g_s on a leaf area basis in spruce, an image was taken of the branchlet that had been within the measuring chamber and the projected leaf area was measured using IMAGEJ 1.49v software (Schneider et al. 2012). To assess the cost of water relative to the carbon gained, we calculated g_1 ($\text{kPa}^{0.5}$; a proxy for the marginal cost of water) by fitting the equation:

$$g_s \cong g_0 + 1.6 \cdot \left(1 + \frac{g_1}{\sqrt{\text{VPD}}}\right) \cdot \left(\frac{A}{Ca}\right) \quad (1)$$

(Medlyn et al. 2011, Hérout et al. 2013). The Ca is the atmospheric CO_2 concentration at the leaf surface ($700 \mu\text{mol mol}^{-1}$). The g_0 (g_s when $A = 0$) was constrained to be non-negative.

Basal area increase and leaf drop

Stem basal diameter was measured on all plants using digital calipers in spring and late summer of 2016 and 2017. A position on the stem (2 cm above the soil) was marked for repeatability of the measurement. Basal area was calculated assuming circularity. Basal area increase was calculated by subtracting the initial stem area in spring from the final stem area in late summer. The treatment means were then adjusted for the initial size of the plant stem by including the initial plant basal area as a covariate in the analysis.

Birch leaf drop was estimated in 2017 because at the time leaf drop was observed in 2016, none of the plants were netted. In the second year of treatment, netting was loosely wrapped around a subset of plants ($n = 4$) in each treatment group. Two weeks after the first and second heatwave events, the dropped leaves were counted then divided by the total leaves produced per plant to get the percent dropped after each heatwave. To estimate leaf drop in the first season of heatwave stress, a subset ($n = 4$) of the non-heated plants (C, D) were netted and moved to the heatwave rooms for the duration of the second growing season. All plants were the same age in 2017 when leaf drop was estimated. These plants were not used for any

other measurements. We did not observe needle loss in 2016 from any of the spruce plants, and therefore, we did not set up catchments around the plants in 2017.

Photosynthetic and respiratory temperature response

Temperature response curves were generated on four to six plants from each treatment group in September 2016 and June, July, and late August 2017 within 2 weeks of the preceding heatwave to determine if acclimation of A occurred. For a given curve, six to eight measurements of A were recorded between 9:00 and 13:00 h at several leaf temperatures ($18\text{--}35 \text{ }^\circ\text{C}$) on a single leaf using the gas exchange settings listed above except that leaf temperatures were altered by adjusting the block temperature. At each sampling date, a different set of plants were randomly selected. For each curve, the maximum net photosynthetic rate (A_{opt}), the temperature at A_{opt} (T_{opt}) and the sharpness of the optimum (b) were estimated by fitting a parabolic function (Eq. 2) to the data. With nonlinear regression using the Excel Solver function:

$$A(T) = A_{\text{opt}} - b(T - T_{\text{opt}})^2 \quad (2)$$

Acclimation of photosynthesis requires (i) the adjustment of T_{opt} and A_{opt} and/or (ii) altering b (Figure 1; Way and Yamori 2014).

Mitochondrial respiration was measured at night (R_{dark}) using the *Set Temperature Method* (Loveys et al. 2003) before and during the final heatwave in August 2017 between 21:00 and 23:00 h on the same leaf on six plants per treatment. Respiration was measured before the heatwave at leaf temperatures of $16\text{--}17 \text{ }^\circ\text{C}$ (average night temperature between 21:00 and 6:00 not during heatwave events). During the heatwave, R_{dark} was measured at leaf temperatures of $24\text{--}25 \text{ }^\circ\text{C}$ (the average night temperature between 21:00 and 6:00 during a heatwave event) for all treatment groups. To reach $24\text{--}25 \text{ }^\circ\text{C}$, plants growing in non-heatwave rooms were transferred to the heatwave rooms 1 h before measurement. Acclimation was assessed by calculating acclimation ratios based on the *Set Temperature Method* ($\text{Acclim}_{\text{Set Temp}}$) and the *Homeostasis Method* ($\text{Acclim}_{\text{Homeo}}$; Loveys et al. 2003). The $\text{Acclim}_{\text{Set Temp}}$ ratio is the ratio of R_{dark} of the control plants divided by R_{dark} of the heated plants measured at a set temperature (i.e., $24\text{--}25 \text{ }^\circ\text{C}$). Values > 1 indicate R_{dark} has acclimated; the greater the value, the greater the degree of acclimation. The $\text{Acclim}_{\text{Homeo}}$ is the ratio of R_{dark} of the control plants at a non-heated temperature (i.e., $16\text{--}17 \text{ }^\circ\text{C}$) divided by R_{dark} of the heated plants at a heated temperature (i.e., $24\text{--}25 \text{ }^\circ\text{C}$). When $\text{Acclim}_{\text{Homeo}}$ is equal to 1, complete acclimation has been achieved. $\text{Acclim}_{\text{Homeo}} < 1$ indicates incomplete acclimation and $\text{Acclim}_{\text{Homeo}} > 1$ indicates overcompensation. Additionally, R_{dark} at elevated temperatures was compared among treatment groups to determine if prior exposure to heatwaves reduces R_{dark} relative to the controls.

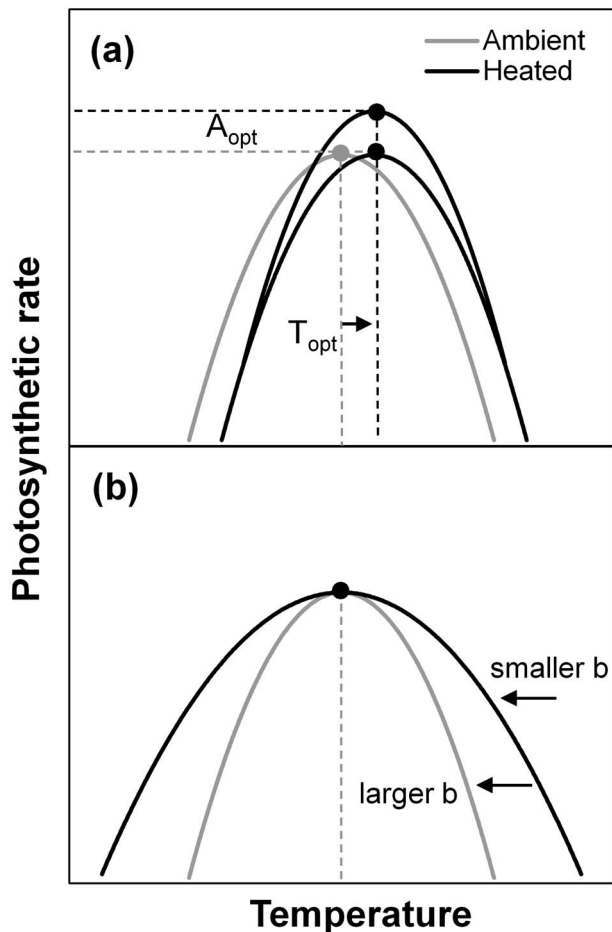


Figure 1. Conceptual representation of thermal adjustment of photosynthesis. Two types of photosynthetic adjustment: (a) a shift of T_{opt} to a higher temperature while maintaining or increasing A_{opt} in the heated plants (modified from Yamori et al. 2014) and (b) the widening of the temperature response curve (b parameter) in heated plants. The gray line represents a plant grown at ambient temperatures; the black line represents a plant grown at elevated temperatures and the dot at the apex of the curve represents the A_{opt} at the T_{opt} .

Reference CO_2 concentrations were set at $700 \mu\text{mol mol}^{-1}$, air flow was set at $200 \mu\text{mol s}^{-1}$ and humidity was not controlled but VPD was typically below 1.5 kPa.

Statistical analyses

The data were analyzed using XLstat 2019 v1.3: Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France) and R (R Core Team 2018). For each species, linear mixed effects models, followed by the Tukey's multiple comparison test, were used. For the analysis of the A , g_s and Ψ_{PD} the fixed factors were *Year* and *Treatment*; the random factors were *Greenhouse room* and *Sampling date nested within year*. For each of the temperature response curves, A_{opt} , T_{opt} and b were estimated by fitting a parabolic function (Eq. 2) to the data with non-linear regression using the Excel Solver function. The analysis of the photosynthetic acclimation data treated *Sampling*

date and *Treatment* as fixed factors and *Greenhouse room* as a random factor. *Treatment* was a fixed factor and *Greenhouse room* was a random factor in the analysis of R_{dark} . This statistical approach was used instead of analyzing a 2×2 factorial design to avoid potential issues of pseudo replication in the first year when there was only one greenhouse room per temperature treatment. To determine if $Acclim_{Set Temp}$ ratios in the treated plants were >1 , one-sample t -tests (one-tailed) were run on the D , H and HD data ($H_0: \mu = 1$ and $H_a: \mu > 1$). To determine if $Acclim_{Homeo}$ ratios in the treated plants were equal to 1, one-sample t -tests (two-tailed) were run on the D , H and HD data ($H_0: \mu = 1$ and $H_a: \mu \neq 1$). An analysis of covariance was performed on the basal area increase data for each species where *initial plant basal area* was included as a covariate (to account for differences in initial plant size) and *Treatment* was the explanatory variable. Linear relationships within a given treatment group between A and g_s , A and Ψ_{PD} and g_s and Ψ_{PD} were determined by linear regression analyses. An analysis of covariance was then performed to determine differences among treatment coefficients on a given date or for a given species. To assess the cost of water relative to the carbon gained (g_1), Eq. (1) was fit using nonlinear least squares (nls() function in R; R Core Team 2018).

Results

Gas exchange, water potentials and growth responses

Gas exchange in both species was strongly affected by treatment ($P < 0.001$; see Table S1 available as Supplementary Data at *Tree Physiology* Online). There was a general decline of A and g_s in the H and HD birch during heatwave events, but in some cases, there was a recovery afterwards (Figure 2). The drought treatment also tended to reduce A and g_s on several occasions and overall resulted in lower seasonal gas exchange rates than the C birch (Figure 2c and d). During both years of growth within birch, the C plants tended to have the greatest values of A and g_s while the HD plants had the lowest. All treatment groups increased A from 2016 to 2017 ($P < 0.001$, $C D HD$; $P < 0.01$, H). Similarly, all treatment groups increased g_s from 2016 to 2017 as well ($P < 0.001$ all treatments). In the first year, the D , H and HD birch had lower g_s than the C plants, but in 2017, only the D and HD plants displayed lower g_s . All treatment groups experienced a decline in A and g_s with decreasing Ψ_{PD} , but no differences were found between the plants that were heatwave stressed (H and HD) and those that were not (C and D ; see Figure S5a and b available as Supplementary Data at *Tree Physiology* Online). The heatwave-treated plants experienced more negative Ψ_{PD} , yet their response in A or g_s did not change. Spruce did not respond as strongly as the birch to the heatwave events (Figure 3). Instead, low water availability appeared to have greater influence on gas exchange in spruce in the first year, especially after

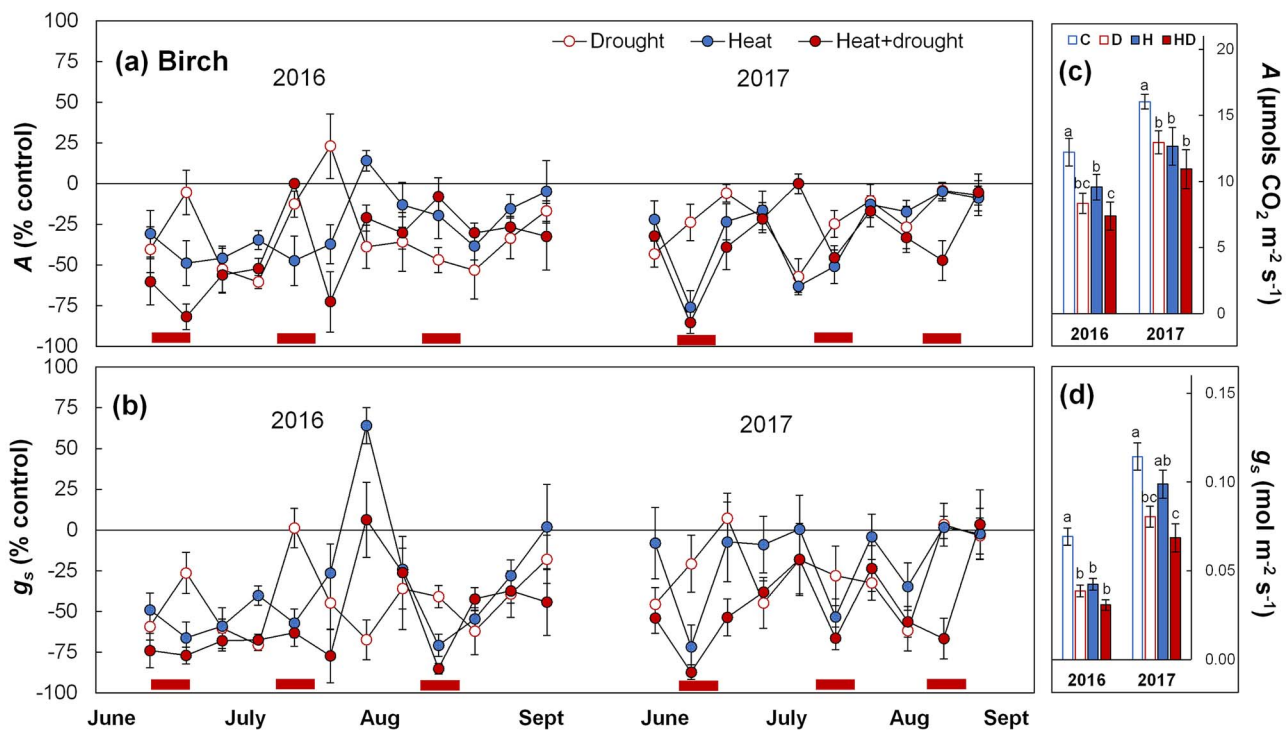


Figure 2. Net photosynthetic rate (A) \pm standard error (SE) (a) and stomatal conductance (g_s) \pm SE (b) for paper birch during the 2016 and 2017 growing seasons. Treatment values are expressed as a percentage of the control values. The red bars indicate heatwave events. Yearly means \pm SE for A and g_s are shown in panels (c) and (d). Different letters above treatment means indicate significant differences ($P < 0.05$) among treatment groups in a given year based on Tukey's HSD post hoc test.

the July heatwave (Figure 3a and b). Relative to the C spruce in 2016, all treatments reduced A and g_s , but the greatest reduction was found in the HD spruce (Figure 3c). By 2017, there was a lack of statistical evidence of differences in A and g_s among treatment groups. Similar to the birch, A and g_s increased within treatment groups from 2016 to 2017 (A : $P < 0.001$ all treatments and g_s : $P < 0.001$ all treatments). There was also a significant decline in A with decreasing Ψ_{PD} , but no differences were found between the plants that experienced heatwave stress (H and HD) and those that did not (C and D ; see Figure S5c available as Supplementary Data at *Tree Physiology Online*). A significant relationship between g_s and Ψ_{PD} was found in the plants that were heatwave-treated but not within the non-heated plants (see Figure S5d available as Supplementary Data at *Tree Physiology Online*). Despite the heatwave-treated plants experiencing more negative Ψ_{PD} and greater stress, their response in A to declining Ψ_{PD} did not differ from the non-heated plants (see Figure S5c available as Supplementary Data at *Tree Physiology Online*).

The slope of A/g_s decreased from 2016 to 2017 across treatment groups in birch (Figure 4a and b). In 2016, no differences in the slopes of A/g_s were found among the treatments, but in 2017, the C treatment had a much shallower slope compared to the H ($P < 0.01$) and HD treatments ($P < 0.01$; Figure 4b). The greatest slope value in birch was found in 2016

in the HD plants ($185 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$), whereas the shallowest slope was found in the C plants in 2017 ($26 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$; Figure 4a and b). In spruce, there was not a significant relationship between A and g_s within any of the treatment groups in 2016 (Figure 4c). In 2017, A/g_s slopes of spruce were $39\text{--}50 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, which were generally lower than in birch but not statistically different from one another (Figure 4d). Additionally, the metric g_1 was greater in both species in 2017 than 2016 indicating a greater degree of water conservation among plants in 2016 (see Figure S6 available as Supplementary Data at *Tree Physiology Online*). In 2016, the droughted birch (D and HD) had lower g_1 values compared to the well-water plants (C and H), whereas in 2017, all treatment groups had similar values. In spruce, g_1 values were $< 1 \text{ kPa}^{0.5}$ in 2016 with no statistically significant differences found among treatment groups. In 2017, g_1 values increased in all treatment groups, but the H plants had the highest (least conservative) g_1 value (see Figure S6 available as Supplementary Data at *Tree Physiology Online*).

Predawn water potentials tended to be more variable in 2016 than in 2017 in both species (Figure 5). In 2016, the HD treatments consistently experienced more negative Ψ_{PD} over the growing season compared to the C plants, whereas Ψ_{PD} of the D and H treatment groups were either similar or more negative than the C plants on a given day (Figure 5a and b). Seasonal

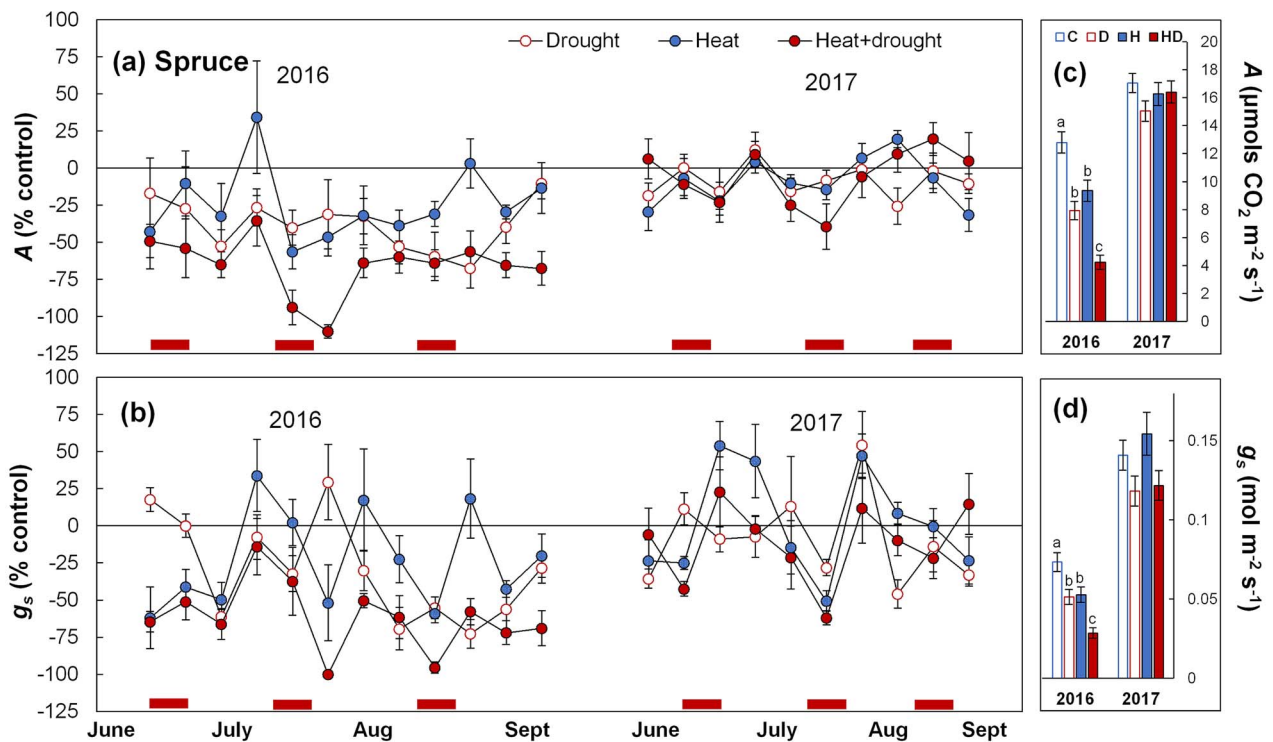


Figure 3. Net photosynthetic rate (A) \pm SE (a) and stomatal conductance (g_s) \pm SE (b) for white spruce during the 2016 and 2017 growing seasons. Treatment values are expressed as a percentage of the control values. The red bars indicate heatwave events. Yearly means \pm SE for A and g_s are shown in panels (c) and (d). Different letters above treatment means indicate significant differences ($P < 0.05$) among treatment groups in a given year based on Tukey's HSD post hoc test.

means in 2016 were lowest in the *HD* plants (-0.54 mPa in birch and -0.84 mPa in spruce) compared to the *C* plants (Figure 5c and d; $P < 0.001$, birch *C* vs *HD*; $P < 0.001$, spruce *C* vs *HD*). The *D* birch also had a lower seasonal Ψ_{PD} mean than the *C* birch at this time ($P < 0.01$, *C* vs *D*). No statistical difference in seasonal means was observed between the *D* and *C* spruce in 2016. In 2017, there was less temporal variability in Ψ_{PD} (excluding June 21st and August 10th), and values were generally less negative for both species ($P < 0.001$, 2016 vs 2017). Seasonal means were similar in the well-watered birch (*C* and *H*) across both seasons, whereas the reduced-watered birch treatments increased Ψ_{PD} from 2016 to 2017 and these treatments no longer differed from the *C* plants. Unlike the birch, all spruce treatment groups increased Ψ_{PD} in 2017, but the *HD* treatment still had the most negative values compared to the *C* treatment ($P < 0.001$, *C* vs *HD*).

Basal area increase in the birch was nearly double that of the spruce for both years (Figure 6). There was a strong effect of treatment in both years for spruce ($P < 0.001$; see Table S2 available as Supplementary Data at *Tree Physiology* Online), but for birch, the effect of treatment was only found in 2016 ($P < 0.001$; see Table S2 available as Supplementary Data at *Tree Physiology* Online). Basal area increase in the *D*, *H* and *HD* birch was significantly lower than the *C* plants in 2016

($P < 0.001$, *C* vs *D*; $P < 0.05$, *C* vs *H*; $P < 0.01$, *C* vs *HD*), but in 2017, growth among the treatment groups was not statistically different (Figure 6a and b). In spruce, basal area increase was not affected by the *D* or *H* treatments in 2016 (Figure 6c). Only the *HD* treatment impaired growth relative to the *C* plants in the first season ($P < 0.001$; Figure 6c). In 2017, low water availability impacted basal area increase where both the *D* and *HD* treatments resulted in lowered growth compared to the *C* plants ($P < 0.001$, *C* vs *D*; $P < 0.001$, *C* vs *HD*; Figure 6d).

Leaf drop occurred in birch in response to heatwave exposure. After the first year of heatwave exposure the birch lost 26% (*H*) and 67% (*HD*) of their leaves. By the second year, there was only 15% (*H*) and 14% (*HD*) leaf loss (see Table S3 available as Supplementary Data at *Tree Physiology* Online) indicating some acclimation occurred by the second year. By the second year of heatwave exposure, the plants lost significantly fewer leaves than what was lost in the first year ($P < 0.05$, *HD* first year vs *HD* second year). Additionally, by the end of the first season, 11% (birch) and 31% (spruce) of *HD* plants died while all other plants survived, except one *C* spruce (data not shown). Only one plant had died after exposure to the first heatwave; 4 additional plants died after the second heatwave and another 10 died by the end of the summer.

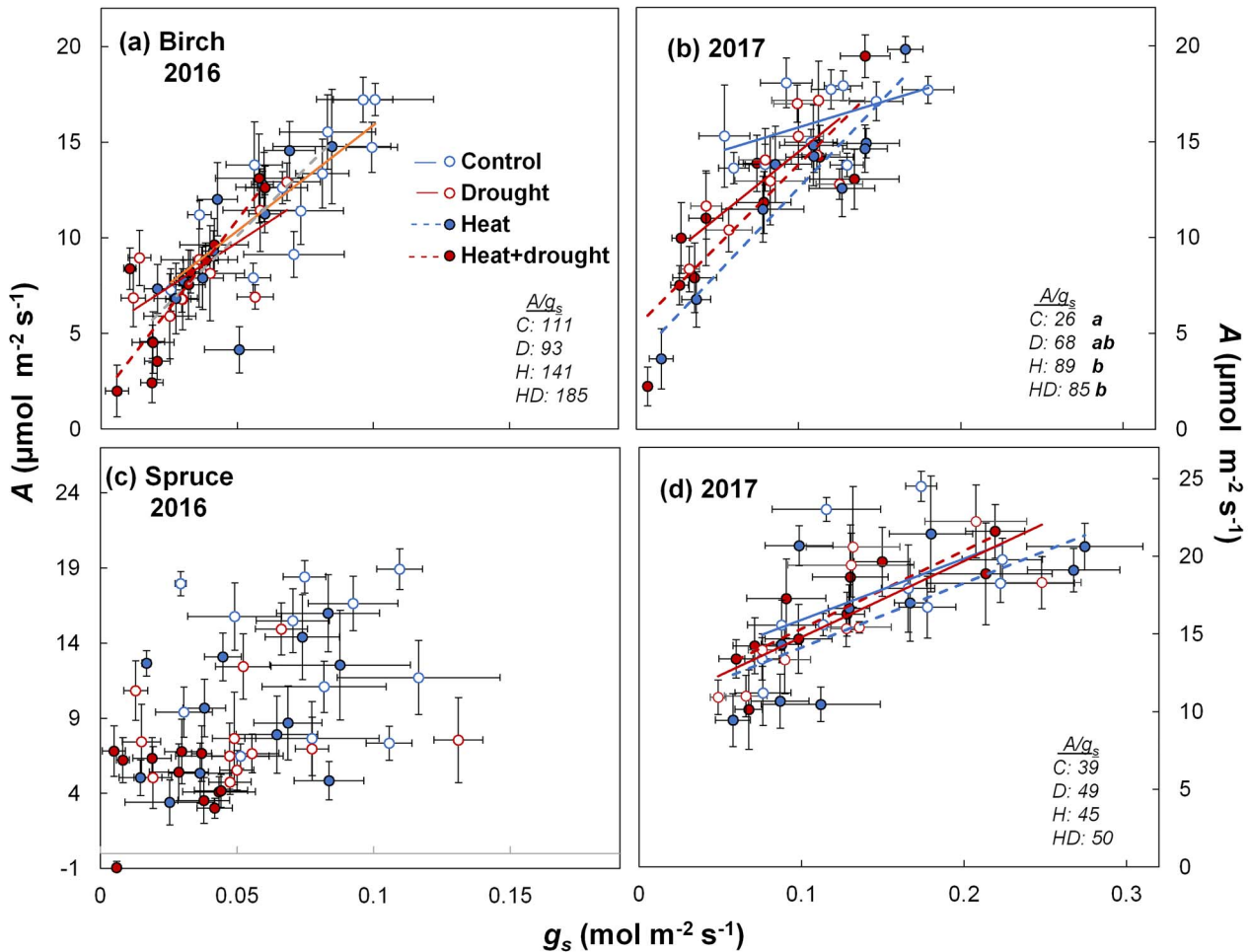


Figure 4. Relationship between photosynthetic rate (A) and stomatal conductance (g_s). Data are of birch (a, b) and spruce (c, d) during the 2016 (a, c) and 2017 (b, d) growing seasons. Each data point represents the mean \pm SE for a given sampling date. All relationships in birch were significant ($P < 0.05$) except for the controls in 2017 ($P = 0.1$). In spruce, relationships were found in 2017 ($P < 0.05$ for D , H , HD ; $P = 0.09$ for C) but not in 2016. The slope of A/g_s ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) for each treatment group is given in the bottom right of each panel. Letters after A/g_s values in panel b indicate differences in A/g_s among treatment groups; the H and HD treatments have steeper slopes than the C treatment ($P < 0.01$ and $P = 0.01$, respectively).

Thermal acclimation of photosynthesis and dark respiration

There was no evidence of photosynthetic acclimation in birch. The T_{opt} and A_{opt} did not increase in response to repeated exposure to heatwave events (Figure 7; see Table S4 and Figure S7 available as Supplementary Data at *Tree Physiology* Online). There were no statistically significant differences in T_{opt} among treatment groups at any of the sampling dates, but T_{opt} did increase over the season in birch ($P < 0.001$, June 2017 vs late Aug 2017). Differences in A_{opt} among the birch treatments were only found in June 2017 where the HD plants had a lower A_{opt} than the C plants ($P < 0.05$; Figure 7b). Additionally, the b parameter was only influenced by sampling date and not by treatment ($P < 0.05$; see Tables S4 and S6 available as Supplementary Data at *Tree Physiology* Online). Unlike birch, spruce showed evidence of minor photosynthetic

acclimation. Both the T_{opt} and A_{opt} were influenced by sampling date ($P < 0.001$, T_{opt} ; $P < 0.001$, A_{opt}) and treatment*date ($P < 0.05$, T_{opt} ; $P < 0.05$, A_{opt} see Table S5 available as Supplementary Data at *Tree Physiology* Online). After the first heatwave in 2017, the H and HD plants that experienced the heatwave had higher T_{opt} than the non-heatwave C and D plants ($P < 0.01$, C vs H ; $P < 0.001$, C vs HD ; $P < 0.01$, D vs H ; $P < 0.001$, D vs HD Figure 7c; see Figure S7 available as Supplementary Data at *Tree Physiology* Online). However, by the end of the 2017 season, no statistically significant differences in T_{opt} were found among treatment groups. There was a seasonal change in T_{opt} where the T_{opt} increased from June to late August ($P < 0.001$, June 2017 vs late Aug 2017). Differences in A_{opt} were only found at the end of the first season where the HD spruce had lower values than the C and H plants ($P < 0.05$, C

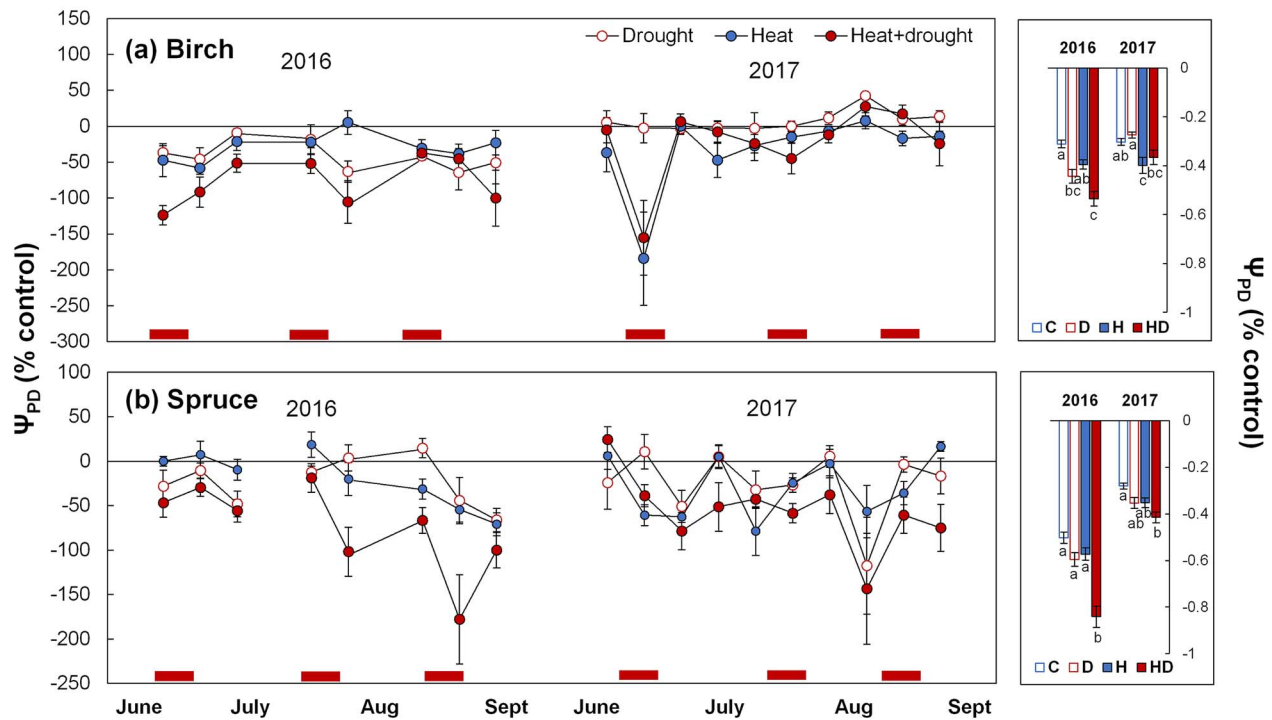


Figure 5. Predawn leaf water potentials (Ψ_{PD}) in birch (a) and spruce (b) across the 2016 and 2017 growing seasons. Each data point represents the mean \pm SE ($n = 5-6$) for a given sampling date. Treatment values are expressed as a percentage of the control values. Yearly means \pm SE for A and g_s are shown in panels (c) and (d). Red bars in panels (a) and (b) indicate heatwave events. Different letters above treatment means indicate significant differences ($P < 0.05$) among treatment groups in a given year based on Tukey's HSD post hoc test.

vs HD; $P < 0.05$, H vs HD; Figure 7d; see Figure S7 available as Supplementary Data at *Tree Physiology* Online). The b parameter was minorly affected by treatment*date ($P = 0.07$), but no statistically significant differences were found among treatment groups at any of the sampling dates (see Table S6 available as Supplementary Data at *Tree Physiology* Online).

Mitochondrial respiration did acclimate in the heatwave-treated birch but not the spruce (Figure 8). In birch, the $Acclim_{Set\ Temp}$ ratio was > 1 and the $Acclim_{Homeo}$ ratio equaled 1 in both H, and HD plants indicating R_{dark} acclimated (Figure 8a and b; see Table S7 available as Supplementary Data at *Tree Physiology* Online). At elevated nighttime temperatures, treatment had an effect on R_{dark} ($P < 0.05$) where the H and HD plants had significantly lower R_{dark} than the C plants ($P < 0.05$, C vs H; $P < 0.05$, C vs HD; Figure 8c; see Table S7 available as Supplementary Data at *Tree Physiology* Online). In spruce, the plants showed only minor responses to the heatwaves (Figure 8). Neither the H nor HD plants had an $Acclim_{Set\ Temp}$ ratio > 1 , but their $Acclim_{Homeo}$ ratio did not differ from 1 (Figure 8d and e; see Table S7 available as Supplementary Data at *Tree Physiology* Online). At elevated nighttime temperatures, there were no statistically significant differences found among any of the spruce treatment groups (Figure 8f; see Table S7 available as Supplementary Data at *Tree Physiology* Online).

Discussion

Climate change has been implicated in growth declines, mortality and range shifts in cool northern regions (e.g., Jump et al. 2009, 2010, Ge et al. 2011). For survival under predicted future climate scenarios, adjustments of metabolic processes under high temperatures will surely be a necessary step. In the present study, we investigated plant responses to high temperatures and drought, in the form of repeated heatwave events, under eCO₂ conditions. In regard to our hypotheses, the data show heatwave stress indeed reduced A, g_s and growth most severely when combined with drought, but it did not fully decouple A from g_s . As hypothesized, repeated heatwave exposure did not promote acclimation of both photosynthesis and respiration in either species but to the contrary, the combination of heat and drought did not affect spruce more than it did birch.

Photosynthetic and stomatal responses to heatwaves

Extreme heat has been shown to decouple A and g_s in a variety of tree species (Ameje et al. 2012, Slot et al. 2016, Urban et al. 2017, Drake et al. 2018), but in the present study, we did not find this to be true for either species. The exception being a partial decoupling in the later part of 2016, where the H spruce reduced A while g_s remained similar to that of the untreated plants. Generally, the heatwave-treated birch (and spruce to a lesser extent) reduced both A and g_s

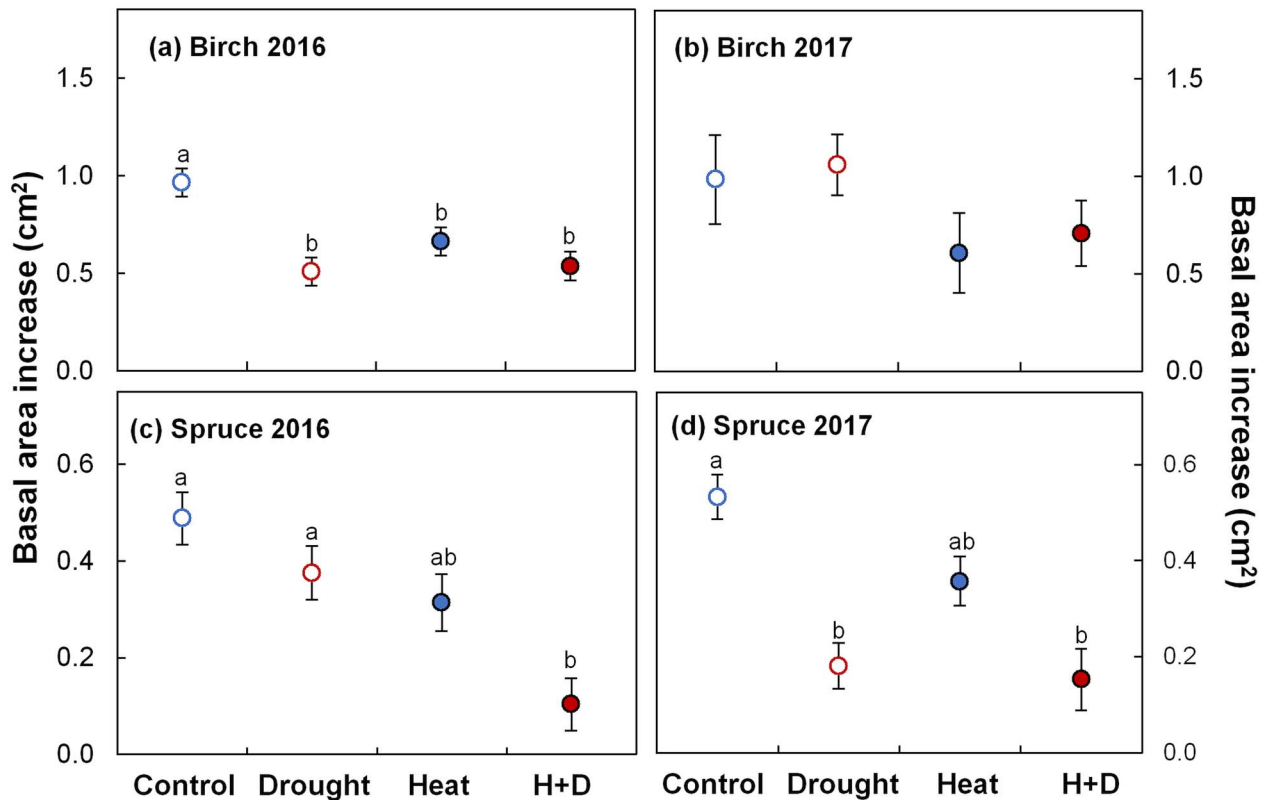


Figure 6. Least squares means of stem basal area increase (cm^2) \pm SE for birch (a, b) and spruce (c, d) in 2016 (left panels) and 2017 (right panels). Stem basal area increase was calculated by subtracting initial stem basal area in spring from the final stem basal area in late summer. Means were then adjusted for the covariate initial plant size. Letters above the means indicate statistical differences among treatment groups within a given panel ($P < 0.05$) based on the Tukey's HSD post hoc test.

proportionally which does not support our second hypothesis. The overall decline in gas exchange in the *H* and *HD* birch is consistent with a water conservation response (Figures 2 and 3; Bauweraerts et al. 2014a, 2014b). The decline of *A* during some heatwave events parallels declines in Ψ_{PD} (e.g., June heatwaves in Figures 2a and 5a). However, the fluctuation of *A* and g_s do not consistently match the fluctuation of Ψ_{PD} which suggests water availability may not have been solely responsible for the reduction of gas exchange in these plants. The response of *A* and g_s to decreasing Ψ_{PD} did not change as stress increased with heatwave exposure, but the heatwaves did drive Ψ_{PD} to become more negative (see Figure S5 available as Supplementary Data at *Tree Physiology Online*). In spruce, the decline in *A* and g_s of the heatwave-treated spruce appears to be driven by soil water availability in 2016 and temperature and VPD in 2017. The response of *A* in the *H* and *HD* spruce mirrors the change in Ψ_{PD} in 2016 suggesting water availability was likely the strongest driver of the decline in the first year of heatwave stress (Figures 3a and 5a). Although declines in gas exchange appear to be driven by water availability in the first season, the heatwave stress did not impact the relationship between gas exchange and Ψ_{PD} (see Figure S5 available as

Supplementary Data at *Tree Physiology Online*). In the second year, however, Ψ_{PD} rarely fell below -0.5 MPa in the *H* and *HD* spruce, yet *A* and g_s still significantly declined during heatwave events which more closely matches fluctuations in VPD (Figure 3; see Figure S4 available as Supplementary Data at *Tree Physiology Online*). There are several potential explanations for the reduction observed in *A*, but carbon limitation is one that can be ruled out. The heatwave-treated plants of both species exhibited non-zero values of g_s under elevated CO_2 conditions indicating that there likely was a sufficient supply of CO_2 within the leaves for carbon fixation. Further, we found that during the warmest heatwave events in July, the treatments with low g_s tended to have lower *A* and C_i (internal CO_2 concentration), but in all cases, $C_i > 250 \mu\text{mol mol}^{-1}$ (data not shown). These values were well above the CO_2 compensation point even in elevated temperatures (e.g., Walker and Cousins 2013). This implies a lack of carbon availability within the leaf was not responsible for the 40–65% reduction in *A* during heatwaves. There are multiple, non-mutually exclusive explanations for the observed decline in *A* that we cannot rule out. First, leaky membranes may have reduced rates of photochemical reactions due to the relationship between *A* and electron transport

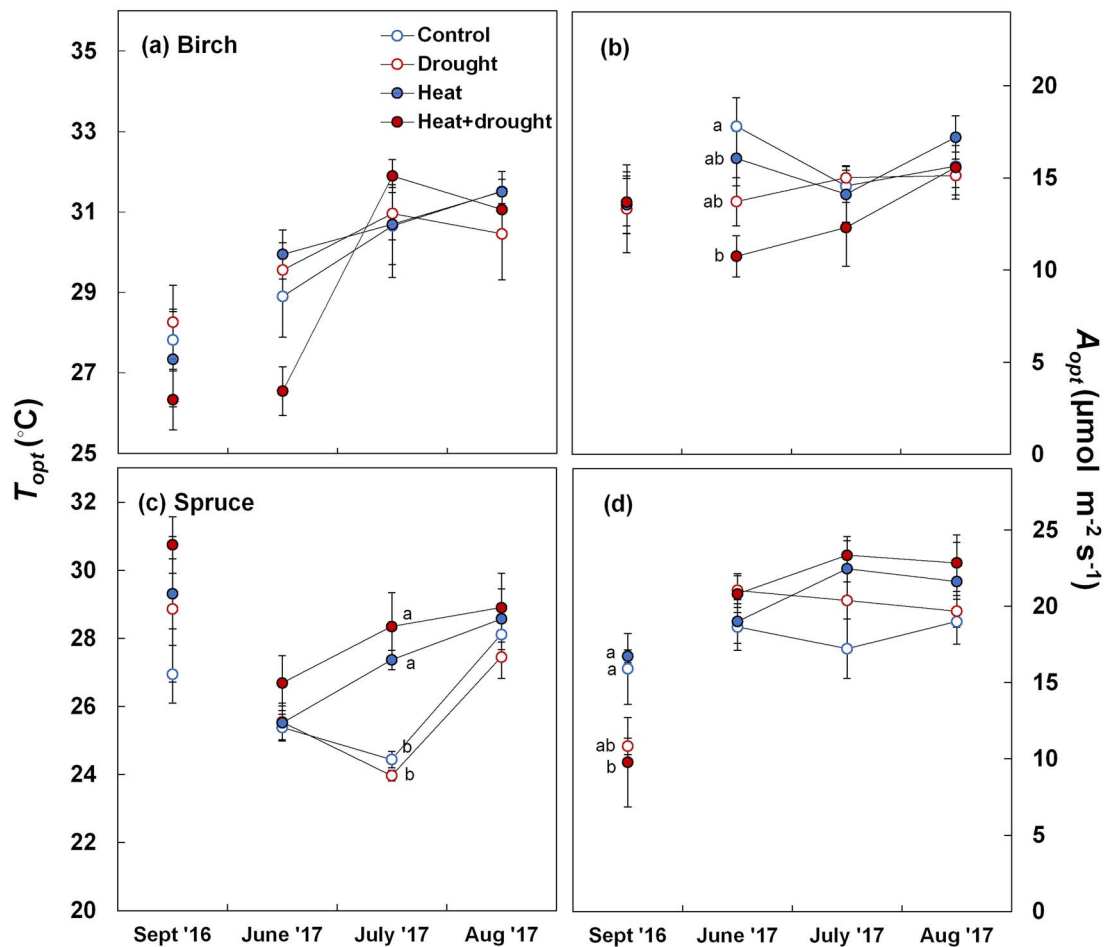


Figure 7. Temperature optima of photosynthesis ($T_{opt} \pm \text{SE}$; a, c) and the photosynthetic optima at $T_{opt} \pm \text{SE}$ (A_{opt} ; b, d) in paper birch (top panels) and white spruce (bottom panels) in September 2016, June 2017, July 2017, and late August 2017. Letters above the means indicate statistical differences ($P < 0.05$) among treatments on a given date on the Tukey's HSD post hoc test.

rate (data not shown). Second, increased photorespiration, resulting from the increased solubility ratio of O_2/CO_2 at high temperatures or decreased RUBISCO specificity for CO_2 also may have reduced A . However, in $e\text{CO}_2$ conditions, increased photorespiration is unlikely (Ku and Edwards 1977, Jordan and Ogren 1984). Third, it is possible that high temperatures decreased RUBISCO activation, which has been shown to occur in $e\text{CO}_2$ and at temperatures as low as 30°C (Feller et al. 1998, Crafts-Brandner and Salvucci 2000). Although we were not able to determine the specific mechanisms responsible for declines in A , it is likely that all of these potential limitations contributed to reducing A during heatwave events.

The reduction of g_s in the heatwave-treated birch plants (H , HD) was somewhat unexpected. Responses of g_s to heat are variable across species. For instance, transpiration increased during periods of extremely high temperatures in *Eucalyptus parramattensis* to maintain or lower leaf temperatures (Drake et al. 2018), but under milder temperature increases, g_s declined in *Alchornea glandulosa* (Fauset et al. 2019).

A species' position on the isohydric-anisohydric spectrum of stomatal regulation may largely determine its response to high temperatures, especially if accompanied with increasing VPD. Birch fall on the isohydric side of the spectrum, and during heatwave events, they consequently tended to show reductions in both A and g_s that loosely mirrored increases in VPD (see Figure S4 available as Supplementary Data at *Tree Physiology Online*). Heatwaves led to higher A/g_s in birch, also contrary to what we expected. Perhaps the environmental or molecular signals responsible for opening stomata at high leaf temperatures were masked by stronger signals from high VPD or $e\text{CO}_2$ to close (Ainsworth and Rogers 2007, Leakey et al. 2009). The birch responded to the low-water conditions in the first year by reducing the marginal water cost of carbon (g_1) values as would be expected for humid environment species (Hérout et al. 2013). By the second season, however, the g_1 values were not different in the low- and well-watered treatments suggesting the increase in g_1 in low-watered plants may have been driven by improved plant-water status (Figure 5) or

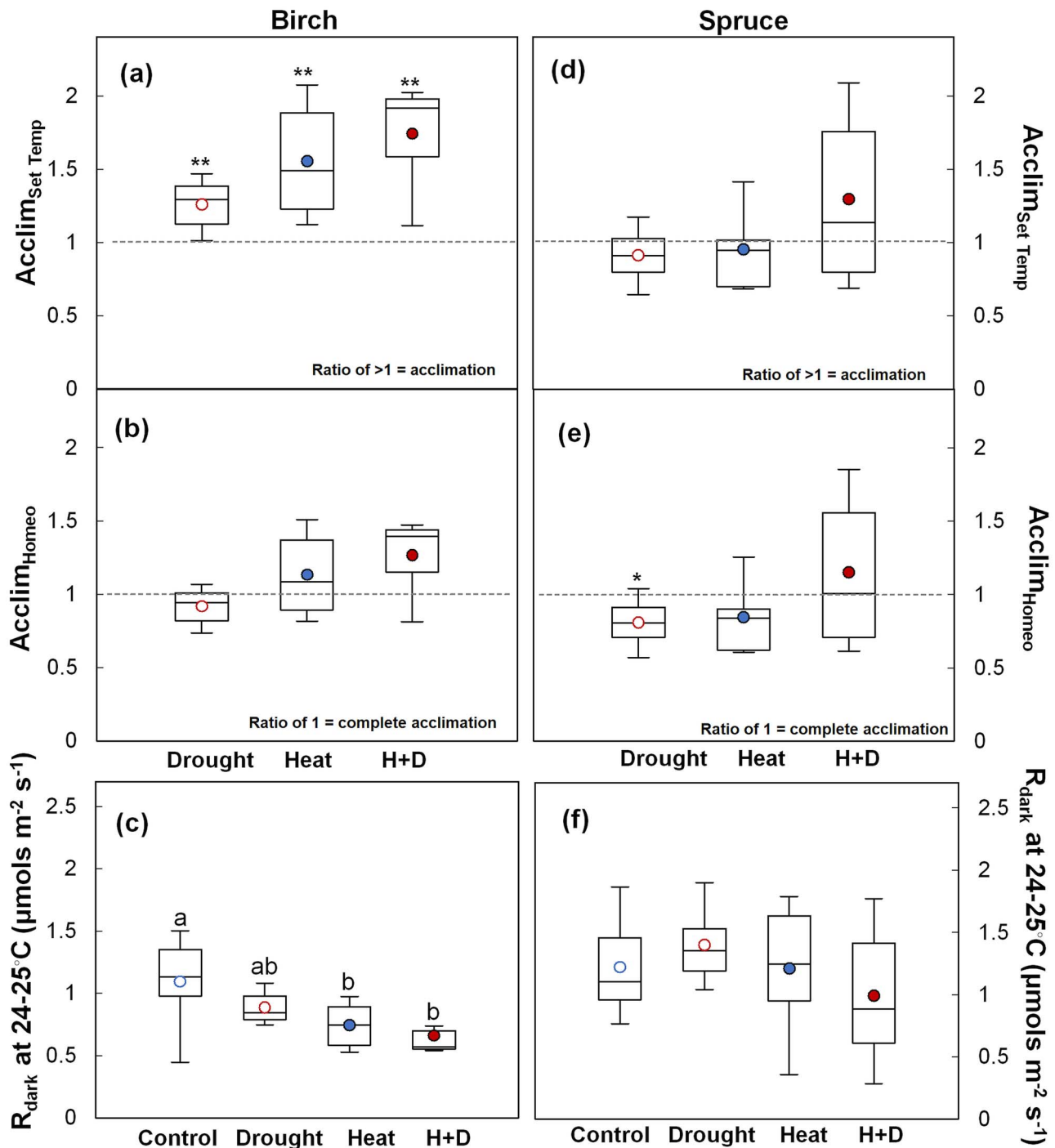


Figure 8. Dark respiration (R_{dark}) in paper birch (a–c) and white spruce (d–f) in August 2017. Acclimation was assessed by calculating acclimation ratios based on the *set temperature method* ($Acclim_{Set\ Temp}$; panels a, b) and the *homeostasis method* ($Acclim_{Homeo}$; panels c, d; Loveys et al. 2003). The $Acclim_{Set\ Temp}$ ratio is the ratio of the R_{dark} of the control plants divided by R_{dark} of the heated plants both measured at 24–25 °C. The greater the value, the greater the degree of acclimation. The $Acclim_{Homeo}$ is the ratio of R_{dark} of the control plants at 16–17 °C divided by R_{dark} of the heated plants at a 24–25 °C. When $Acclim_{Homeo}$ is equal to 1, complete acclimation has been achieved. $Acclim_{Homeo} < 1$ indicates incomplete acclimation and $Acclim_{Homeo} > 1$ indicates overcompensation. Box plots represent the mean (circle symbol), median, first and third quartiles and the whiskers extend to 1.5 times the interquartile range. The dashed line in panels a, b, d and e represents an acclimation ratio of 1 indicating full acclimation or homeostasis. Asterisks in panel (a) indicate $Acclim_{Set\ Temp} > 1$; asterisks in panel (e) indicate $Acclim_{Homeo} \neq 1$ (** $P < 0.01$; * $P = 0.05$). Letters in panel c indicate statistical differences ($P < 0.05$) among treatments based on the Tukey's HSD post hoc test.

perhaps a change in stomatal behavior from the first to second year. The spruce plants also had improved plant–water status in 2017 which may have contributed to g_s values similar to that of the controls. Higher g_s prevented leaf temperatures from rising above air temperature (data not shown), which may have enabled A during periods of high temperatures (Figure 3a). Additionally, eCO₂ conditions may have allowed for non-zero values of A at extremely low values of g_s . Contrary to what was expected for humid environment species under low-water conditions, g_1 values in the low-water spruce were not lower than the well-watered plants in either season. This response was particularly unexpected for the HD spruce because their Ψ_{PD} was consistently more negative than the well-watered plants (Figure 5). The effects of eCO₂ may have interfered with the typical stomatal behavior response to low-water conditions leading to an irregular response of the g_1 parameter (see Figure S6 available as Supplementary Data at *Tree Physiology* Online). Additionally, the different responses in g_s between species may be explained by differences in stomatal sensitivity to VPD or perhaps boundary layer thickness (Johnson et al. 2012, Leigh et al. 2017). Our results are consistent with previous work showing that conifers have a weaker response to VPD than angiosperms (Johnson et al. 2012, Carnicer et al. 2013, Brodribb et al. 2014).

The decline in gas exchange and basal area increase in both species supports our first hypothesis that these variables would be the most affected under the combination of heatwave and drought stress (Figures 2, 3 and 6). The mortality observed in the HD plants of both species in 2016 further supports this hypothesis. In birch, the major reduction in A , coupled with the estimated 67% leaf loss in the HD plants, likely explains the low increase in basal area observed in the first year of treatment. By the second year, however, basal area increase between the HD and C plants was similar although A was still significantly reduced in the HD plants (Figures 2c and 6b). Perhaps because the HD plants had improved plant–water status, which may have maintained 85% of their total leaves and increased A by 48.5% in 2017 relative to 2016, basal area increase was comparable to C plants in the second year. This response coupled with the complete lack of mortality in 2017 in the HD birch could suggest the combination of an improvement in plant–water status and some degree of thermal acclimation attributed to this enhanced response. Alternatively, the difference in growth between years may also be explained by ontogenetic effects. The birch plants were much larger in size in 2017 indicating they likely had a greater pool of storage reserves for use in post-stress recovery which may have been unattainable the previous year. Conversely, in spruce, the response of A cannot explain the decline in basal area increase in both years. A was reduced in the first year but recovered in the second year perhaps due to improved plant–water status, yet these plants still exhibited lower growth in 2017 (Figures 3c and 6d).

Perhaps the combination of high temperatures and drought stress resulted in higher energy costs of cellular maintenance, and therefore, a greater proportion of assimilated carbon may have been allocated to maintenance as opposed to structure. Additionally, the spruce did not increase in size as rapidly as the birch from 2016 to 2017. The relatively small plant size, and consequently small pool of energy reserves in 2017, may explain their inability to recover from stress and increase growth. Similarly, Bauweraerts et al. (2014b) found a significant reduction in stem diameter growth and A when *Pinus taeda* seedlings were exposed to +12 °C heatwave stress. Way and Sage (2008) also found a decrease in biomass in *Picea mariana* grown at +8 °C and attributed the decline to suppression of A and increased daytime respiration. Overall, the 2-year reduction in gas exchange in birch and 2-year reduction in growth of spruce shows that both species are negatively impacted by heatwave stress. These data provide evidence disproving our last hypothesis, which predicted the effects of heatwave stress would be more severe in the conifer.

Thermal acclimation of photosynthesis and dark respiration

We found support for our third hypothesis that exposure to recurrent heatwaves did not promote thermal photosynthetic and respiratory acclimation in either species. Our data, as well as previous work, suggest boreal species may be more sensitive to temperature and therefore lack the capacity for acclimation (Way and Sage 2008, Dillaway and Kruger 2010, 2011, Hozain et al. 2010, Ow et al. 2010, Silim et al. 2010, Zhang et al. 2015). In birch, the lack of photosynthetic acclimation is consistent with some previous findings in *B. papyrifera* (Dillaway and Kruger 2010) but inconsistent with others (Sendall et al. 2014). Ecotype and growth temperatures may have influenced the acclimation potential of the birch in our study. Our seedlings were from a northern Wisconsin population (46.0° N 91.5° W) near the species' southernmost range which could mean the T_{opt} of these plants was already at the species' thermal maximum. Similarly, Dillaway and Kruger (2010) did not find evidence of photosynthetic acclimation in birch from a more southern population (45° N 89° W) than that of the current study. By contrast, the plants in the Sendall et al. (2014) study were from a more northern location (46.7° N 92.5° W) where these plants experienced much lower ambient and elevated temperatures than the current study. The difference in growth temperatures could have led to T_{opt} values that were much lower than the species' maxima in the more northern populations. Upon warming, these plants then had the capacity to adjust physiologically to increase their T_{opt} to reflect the elevated growth temperatures. Consistent with this logic, *Quercus rubra* seedlings and *Quercus serrata* trees growing near their warm temperature limit were restricted in their ability to photosynthetically acclimate T_{opt} to warmer growth temperatures (Wertin et al. 2011, Yamaguchi et al. 2019). These findings provide

a physiological mechanism for the hypothesis that temperature has an influence on the limits of deciduous tree species ranges at the warmest part of their distribution (Bonan and Sirois 1992, Jump et al. 2006, Lesica and Crone 2017).

In contrast to birch, our results suggest that (i) spruce adjusted their T_{opt} seasonally to a higher temperature later in the growing season ($P < 0.001$, June vs August 2017) and (ii) early exposure to high temperatures stimulated an earlier adjustment to the seasonal maximum (Figure 7c, July 2017). We were surprised to find some evidence of thermal photosynthetic acclimation in white spruce seedlings because it has not been shown in other *Picea* species (Way and Sage 2008, Zhang et al. 2015, Benomar et al. 2018, Kurepin et al. 2018). For example, a recent study examining metabolic acclimation of white spruce seedlings from northern and southern populations in Canada found similar T_{opt} (19 ± 1.2 °C) among sites along a latitudinal gradient (Benomar et al. 2018). However, temperatures only differed by a maximum of 5.5 °C among sites, whereas our plants experienced a 10 °C difference. Plant responses are often more affected by heatwaves than by mild temperature increases (Bauweraerts et al. 2013).

Despite limited photosynthetic acclimation, the heatwave-treated birch displayed evidence of respiratory acclimation at elevated nighttime temperatures (i.e., reduced R_{dark} at 24–25 °C and $\text{Acclim}_{\text{Set Temp}} > 1$ and $\text{Accmil}_{\text{Homeo}} = 1$; Figure 8). Lower R_{dark} could be in response to lower A if R_{dark} declined to maintain carbohydrate homeostasis and a constant ratio between A/R (Loveys et al. 2003, Atkin et al. 2006). If the reduction in R_{dark} was to maintain carbohydrate homeostasis, we may have expected to find lower concentrations of non-structural carbohydrates such as soluble sugars. However, the heated plants did not have lower concentrations of glucose, fructose or sucrose at this time (unpublished data). Atkin et al. (2006) showed decreased A/R at high temperatures, whereas homeostasis of A/R was maintained at moderate temperatures suggesting a decoupling of these two processes at extreme temperatures. We did not estimate R_{day} at elevated temperatures and, therefore, it is unclear how R_{day} may have affected the carbon balance. Additionally, drought stress has shown to disrupt respiration in several species (Atkin and Macherel 2009) and may have partially been responsible for the decline of R_{dark} , but since we did not find a significant reduction in R_{dark} of the D plants, it is unlikely drought alone caused the decline in R_{dark} of the H and HD plants. It remains unclear which mechanisms were responsible for reduced R_{dark} in the heatwave-treated birch, but their response of only adjusting R_{dark} and not T_{opt} , A_{opt} or b is quite common (e.g., Zhang et al. 2015, Reich et al. 2016, Benomar et al. 2018, Kurepin et al. 2018).

Unexpectedly, we did not find evidence of thermal respiratory acclimation when measuring R_{dark} at one set temperature (24–25 °C) in white spruce (Figure 8). These findings contrast with several previous studies on conifers that show respiration

is more likely to thermally acclimate than photosynthesis (Ow et al. 2008a, 2008b, Way and Sage 2008, Zhang et al. 2015, Benomar et al. 2018). However, Kurepin et al. (2018) found weak evidence of respiratory acclimation in *Picea abies* and suggest, along with previous studies on this genus, that *Picea* has a limited ability to cope with warming. Similarly, young *P. glauca* grown under warming of +3.4 °C showed a weak response in respiration to warming (Reich et al. 2016). In another *Picea* species, Way and Sage (2008) showed that R_{dark} was similar between warmed and control seedlings, whereas R_{day} was 47% higher in the warm grown plants. It is possible that if we had measured R_{dark} at multiple temperatures or during the day, we may have found differences at higher temperatures (>25 °C).

The mechanisms behind high temperature acclimation of photosynthesis are species dependent and may occur by adjusting different traits (i.e., shifting T_{opt} vs b). It is not clear if there are tradeoffs associated with acclimation via increasing T_{opt} , widening the curve, or adjusting both (Figure 1). Several mechanisms have been proposed that allow for photosynthesis to acclimate to high temperatures, including: improved membrane stability and thermotolerance of the activation state of RUBISCO (Law and Crafts-Brandner 1999, Yamori et al. 2006, Way and Sage 2008), acclimation of heat-labile RUBISCO activase and the activation energy of the maximum rate of RuBP carboxylation (Hikosaka et al. 2006, Weston et al. 2007), altering gene expression of heat shock proteins (Huerta et al. 2013) and of RUBISCO activase isoforms or their ratio (Wang et al. 2010). Which mechanism(s) a species employs would affect the type of photosynthetic acclimation. For example, improving the stability of the thylakoid membranes may result in a wider curve by allowing photosynthesis to remain high across a greater temperature range but may not cause a shift in T_{opt} . Whereas increasing the relative amount of the heat-stable RUBISCO activase isoform may shift T_{opt} but not change the shape of the curve. At this time, it is unclear which mechanisms allow white spruce to acclimate or whether paper birch is unable to adjust the traits listed previously. To our knowledge, there have not been studies that link the different mechanisms of photosynthetic acclimation to the resulting physiological response.

Conclusions

Exposing plants to recurrent heatwave events over two growing seasons allowed for a thorough assessment of plant growth, survival and acclimation that would otherwise not be possible in a short-term, single-event exposure study. This experimental design enabled us to detect the immediate, transient outcomes as well as the long-term consequences of frequent heatwave events. It has also allowed us to examine metabolic adjustments made in response to prior heatwave exposure, i.e., the following season after the initial stress exposure. For example, we

witnessed mortality from heatwave and drought stress in the first year, but not in the second year, a response that would not have been observed in a 1-year study. Additionally, of the plants that died, most did so only after the final heatwave, not after the first or second. This effect would also have been missed in a 1-year or single-event study. We encourage future investigations of species' responses to climatic changes to incorporate repeated treatments that span multiple growing seasons into the experimental design.

Our data show minimal capacity for thermal acclimation in paper birch and white spruce. Although birch adjusted R_{dark} and spruce altered T_{opt} , these minor adjustments were not sufficient in preventing growth declines or reductions in A from two seasons of repeated heatwave and drought exposure. Basal area increase and leaf loss improved in the birch during the second year of treatment and gas exchange in spruce drastically improved as well which may be a reflection of improved water status in the second year. The collective reduction in growth, A , and lack of photosynthetic and respiratory acclimation may act against these species during future heatwave and drought events and eventually minimize their distribution within the boreal forest.

Future investigations into the mechanisms involved in the reduction of A , growth, and the capacity for metabolic acclimation in these species from continuous mild temperature increases vs high temperature heatwaves are needed. With many species exhibiting limited ability to thermally acclimate, understanding the mechanisms involved in acclimation response to high temperature stress is critical to understanding how forests may respond to future climate scenarios.

Supplementary Data

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

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contribution

MG and KM designed the project. MG collected and analyzed the data and wrote the manuscript. DS provided technical support. All authors contributed to revising the manuscript.

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