

Acclimation effects of heat waves and elevated [CO₂] on gas exchange and chlorophyll fluorescence of northern red oak (*Quercus rubra* L.) seedlings

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Abstract Heat wave frequency and intensity are predicted to increase. We investigated whether repeated exposure to heat waves would induce acclimation in *Quercus rubra* seedlings and considered [CO₂] as an interacting factor. We measured gas exchange and chlorophyll fluorescence of seedlings grown in 380 (C_A) or 700 (C_E) μmol CO₂ mol⁻¹, and three temperature treatments (ambient, ambient +3 °C, and an ambient +12 °C heat wave every fourth week). Measurements were performed during the third and fourth +12 °C heat waves (July and August 2010) at Whitehall Forest, GA, USA. Additionally, previously unexposed seedlings were subjected to the August heat wave to serve as a control to determine acclimation of seedlings which were

previously exposed. Seedlings with a history of heat wave exposure showed lower net photosynthesis (A_{net}) and stomatal conductance (on average -47 and -38 %, respectively) than seedlings with no such history, when both were subjected to the same +12 °C heat wave. During both heat waves, A_{net} significantly declined in the +12 °C treatment compared with the other treatments. Additionally, the A_{net} decline during the August compared with the July heat wave was stronger in C_E than in C_A, suggesting that elevated [CO₂] might have had a negative effect on acclimation capacity. We conclude that seedlings subjected to consecutive heat waves will moderate stomatal conductance outside the heat wave, to reduce water usage at lower temperatures, increasing survival at the expense of carbon assimilation.

Ingvar Bauweraerts and Maarten Ameye have contributed equally to this manuscript.

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Introduction

Global climate change is expected to result in a rise in mean global temperature by the end of the century, due in part to a steady rise in atmospheric CO₂ concentration ([CO₂]) and other greenhouse gasses (IPCC 2013). Climate change has already resulted in increases in the number and intensity of heat waves (Rahmstorf and Coumou 2011), a trend considered very likely to continue in this century (Diffenbaugh and Ashfaq 2010; Lynn et al. 2007). Heat waves are attracting public and scientific interest as the number of extreme weather events has increased in recent decades (Coumou and Rahmstorf 2012). Examples include the 2003 European heat wave (Ciais et al. 2005), the 2009 Australian heat wave (Károly 2009), and the 2011 heat wave in the Southern USA (Luo and Zhang 2012). The number of heat events longer than 10 days significantly increased in many regions in Pakistan from 1961 to 2009 (Zahid and Rasul 2012), and the number of summer heat waves in the Eastern USA increased by 20 % from 1949 to 1995 (Gaffen and Ross 1998). Modelling by Coumou and Robinson (2013) has shown that the global land area experiencing temperatures three or more standard deviations warmer than average local climatology will have doubled (covering ~10 %) by 2020 and quadrupled (~20 %) by 2040. In this manuscript, the term “heat wave” denotes a time period during which air temperature is not only significantly higher than its average over the past 30 years, but furthermore high enough to induce a distinct response from the studied object (in this case: tree seedlings). This definition is loosely based on Reichstein et al. (2013), who attempted to give a more general definition of climate extremes.

Changes in heat wave frequency and intensity will not happen overnight, and it is plausible that individuals and ecosystems will acclimate and eventually adapt to their altered thermal environment. Acclimation can be defined as environmentally induced changes in characteristics that result in an improved performance under the new growth regime (Berry and Björkman 1980). Indeed, species have already developed mechanisms to cope with extreme temperatures and heat waves throughout their evolution, and individuals may therefore be able to acclimate to conditions that are more extreme. In this manuscript, we discuss two plant strategies for coping with heat stress, namely thermal acclimation of carbon gain and water conservation. In a

recent review, Way and Yamori (2014) argued that photosynthetic thermal acclimation should include adjustments of both the photosynthetic thermal optimum (T_{opt}) and the photosynthetic rate at the growth temperature (A_{growth}). Many studies have reported an increase in T_{opt} (Sage and Kubien 2007), but A_{growth} has both increased (e.g. *Geum vernum*, Anderson and Cipollini 2013) and decreased (e.g. *Cucumis sativus*, Li et al. 2013). Acclimation to a sustained growth temperature may differ from acclimation to short intense heat events, and plants have been observed to increase expression of heat-shock proteins to correct the structure of heat-damaged photosynthetic proteins during an intense heat period (Vierling 1991). For example, Huerta et al. (2013) found that *Aloe barbadensis* plants acclimated at higher temperatures expressed more heat-shock proteins prior to a 45 °C heat shock than nonacclimated individuals.

Acclimation of a plant's water status to higher temperatures has been studied less extensively, and no specific literature exists on the mechanistic background of water strategy responses to heat waves. The general assumption remains that individuals will strive to conserve water at higher temperatures by reducing stomatal conductance (Fischer et al. 1970) or adjusting osmotic balance (Lu and Zhang 1999), until potentially damaging temperatures and the need for evaporative cooling become more important than water conservation. In a heat wave experiment on *Quercus rubra* seedlings, Bauweraerts et al. (2013) observed that seedlings with a history of >50 °C heat wave exposure had greatly increased stomatal conductance, transpiration, and net photosynthesis (A_{net}) during a >50 °C heat wave compared with measurements before and after the heat wave. In the same experiment, Ameye et al. (2012) found no such behaviour in these seedlings 1 month (and one >50 °C heat wave) earlier.

It has been shown that elevated [CO₂] has the potential to stimulate A_{net} at higher temperatures and mitigate damage at very high temperatures (>45 °C) (Sage and Kubien 2007). In a heat wave experiment on *Larrea tridentata*, Hamerlynck et al. (2000) found that elevated [CO₂] protected photosynthetic activity and capacity during the heat wave. Bauweraerts et al. (2014), in their heat wave experiment, found that biomass of *Q. rubra* seedlings grown under elevated [CO₂] (700 μmol CO₂ mol⁻¹) was not different between ambient +3 and +12 °C heat wave treatments, while biomass of seedlings grown under

ambient $[\text{CO}_2]$ was 40 % lower in the $+12\text{ }^\circ\text{C}$ heat wave treatment compared with the ambient $+3\text{ }^\circ\text{C}$ treatment.

In this report, we examine the effect of consecutive severe ($+12\text{ }^\circ\text{C}$) heat waves on gas exchange and chlorophyll *a* fluorescence of *Q. rubra* seedlings. Data were collected during the third and fourth $+12\text{ }^\circ\text{C}$ weeklong heat waves (July and August). Part of this dataset (gas exchange and chlorophyll *a* fluorescence data from July, and afternoon gas exchange data from August) has been published in Ameye et al. (2012) and Bauweraerts et al. (2013), where it was used to compare the response of two species (*Q. rubra* and *Pinus taeda*) to one heat wave. The current report compares these data to unpublished values recorded in August, to identify signs of acclimation in the evolution of a single species' response to successive heat waves. Additionally, during the fourth heat wave, putative acclimation of physiology after repeated heat wave exposure is tested against controls without previous exposure. Elevated $[\text{CO}_2]$ is considered as a potential interacting factor in the framework of climate change. Our initial hypotheses with regard to the August acclimation experiment were as follows: (1) seedlings with a history of heat wave exposure will have higher A_{net} and Φ_{PSII} than seedlings with no history of heat wave exposure when they are subjected to the same heat wave; (2) elevated $[\text{CO}_2]$ will mitigate differences in A_{net} and Φ_{PSII} between seedlings with a history of heat wave exposure and seedlings with no history of heat wave exposure when they are subjected to the same heat wave. Our hypotheses with regard to the consecutive July and August heat waves experiment were as follows: (3) the difference in A_{net} and effective PSII quantum yield (Φ_{PSII}) between the severe heat wave treatment and the ambient temperature treatment will be smaller, because of acclimation, in the August heat wave than in the July heat wave; (4) elevated $[\text{CO}_2]$ will mitigate differences in A_{net} and Φ_{PSII} among the temperature treatments during both heat waves.

Materials and methods

Study site

The study site was located at Whitehall Forest, a research unit of the University of Georgia in Athens, GA ($33^\circ57'\text{N}$, $83^\circ19'\text{W}$, altitude 230 m). Six treatment

chambers, half-cylinder in shape and measuring 3.62 m length by 3.62 m width by 2.31 m height were constructed at the site. The chambers were constructed according to the method described in Boyette and Bilderback (1996), with lumber bases and PVC pipe frames supporting 0.15-mm-thick clear polyethylene film (GT Performance Film, Green-Tek Inc., Edgerton, WI, USA). The chambers were placed in an open field, spaced 3.7 m apart to minimise shading, and oriented facing south to maximise daily sun exposure.

Plant material

Seeds of *Quercus rubra* L. were planted on 1 December 2009 in 0.5-L pots in potting medium (Fafard Nursery Mix, Conrad Fafard Inc., Agawam, MA, USA). The seeds were obtained from a wild collection in Tennessee (Louisiana Forest Seed Company, 135 Lecompte, LA, USA). This provenance is in the southernmost region of the species range (Little 1971).

In February 2010, the seedlings were transplanted to 8 L pots (cylindrical in shape; 22 cm diameter, 22 cm height) in the same potting medium. The pots were watered three times a day to saturation until treatments started. In April, May, June, and July, each pot was fertilised with 30 g of 15-9-12 extended release fertiliser (Osmocote plus #903286, Scotts-Sierra Horticultural Products, Marysville, OH, USA). In May, 0.04 mL Imidacloprid was applied topically to the soil in each pot to prevent insect infestation (Bayer Advanced 12 months tree and shrub insect control, Bayer, Monheim am Rhein, Germany).

Experimental design and monitoring

The setup of this experiment was based on Wertin et al. (2010). Each treatment chamber was assigned one of six treatment combinations. These combinations were produced by two factors: atmospheric $[\text{CO}_2]$ and chamber temperature. The $[\text{CO}_2]$ treatments were ambient $[\text{CO}_2]$ (C_A , $380\text{ }\mu\text{mol CO}_2\text{ mol}^{-1}$) or elevated $[\text{CO}_2]$ (C_E , $700\text{ }\mu\text{mol CO}_2\text{ mol}^{-1}$). The temperature treatments were ambient (T_{A0}), ambient $+3\text{ }^\circ\text{C}$ (T_{A3}), and a heat wave elevating ambient by $12\text{ }^\circ\text{C}$ every fourth week ($T_{\text{HW}12}$). Each applied heat wave lasted for 1 week, and the heat wave treatment scheme was repeated every 4 weeks throughout the growing season

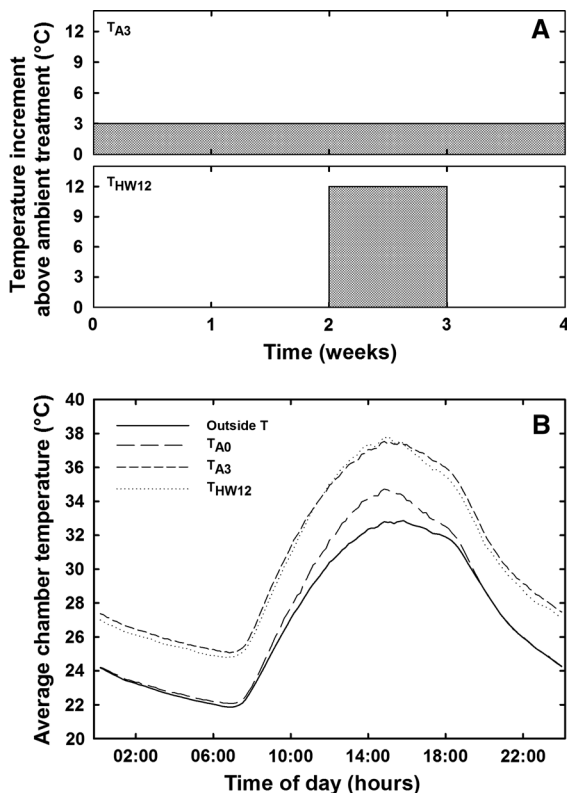


Fig. 1 **a** Chamber temperature treatment scheme for the elevated temperature treatments T_{A3} and T_{HW12} . While T_{A3} is set to constantly raise chamber temperature 3 °C above the ambient temperature treatment T_{A0} , T_{HW12} is set to raise chamber temperature 12 °C above T_{A0} for 1 week during every 4-week period. *Shaded areas* represent the temperature increment above the T_{A0} temperature. The *two shaded areas* are of equal size, meaning that the average temperature increments over the 4-week period are equal. **b** Average diurnal temperature profile in the chambers at three temperature treatments T_{A0} , T_{A3} , and T_{HW12} , as well as outside of the treatment chambers over the course of the entire experiment from 2 May through 2 October (156 days). Temperatures were measured 1 m above the ground

(Fig. 1a). The elevated temperature treatments T_{A3} and T_{HW12} represented the same applied heat sum and corresponded to an average increase of 3 °C over a 4-week period and the entire growing season (May through September). In this context, the heat treatments may also be considered as differences in temperature variability. Thus, the experiment was a complete factorial of 2 [CO_2] \times 3 temperature treatments.

Fifteen *Q. rubra* seedlings were placed in each chamber. The seedlings were randomly assigned to one of three blocks per species per chamber. Pots were evenly spaced within the chamber. To minimise any

potential chamber effect, the treatment combinations, blocks, and pots within blocks were randomly rotated among the chambers every 4 weeks, and chamber CO_2 and temperature conditions were adjusted accordingly. Treatments were initiated on 2 May 2010.

To maintain [CO_2] at the desired levels, a nondispersive infrared CO_2 -sensor (Model GMT222, Vaisala Inc., Woburn, MA, USA) continuously measured [CO_2] inside each chamber and directly controlled a solenoid valve which released CO_2 into the chambers as necessary from a cylinder of industrial grade compressed 100 % CO_2 (Airgas National Welders, Toccoa, GA, USA). An oscillating fan was installed in each chamber to disperse the CO_2 evenly throughout the chamber. A datalogger (23X, Campbell Scientific, Logan UT, USA) was used to monitor air temperature with a thermocouple inside each chamber and outside 1.45 m south of the chambers. Each thermocouple was housed in a ventilated radiation shield (Model SRS100, AmbientWeather, Chandler, AZ, USA) mounted on a pole 1 m above ground level. The datalogger was programmed to compare chamber air temperature with outside temperature at a 1-min interval, and control air conditioners (FAM186R2A, Frigidaire, Augusta, GA, USA) and resistance heaters (3VU33A, Dayton Electric, Niles, IL, USA) to maintain the treatment target air temperatures. Air conditioners and resistance heaters were controlled using a simple on/off signal, meaning that there was some lag and overshoot in the temperature control, though this effect was negligible.

Soil water content in the pots was measured with soil moisture probes (ECH2O EC5, Decagon Devices, Pullman, WA, USA), which were inserted to a depth of 9 cm, and recorded every 10 min with the datalogger. Probes were inserted in two pots per chamber. To maintain the soil at field capacity (ca. 51 %), plants were provided with three watering events of 8 min per day, during each of which 84 mL (=252 mL in total) was applied via an automated irrigation system and drip emitters (Supertif—PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). Drip emitters were attached right above the soil surface, on the opposite side of the pot from the soil moisture sensors.

From 2 May to 2 October 2010, ambient temperature outside the chambers averaged 29.5 °C. Over the same time period, the elevated temperature treatments T_{A3} and T_{HW12} averaged 3.2 and 2.9 °C, respectively, above the ambient temperature treatment T_{A0} (Fig. 1b). Mean

Table 1 Mean (SE) air temperature (°C) in the environmentally controlled treatment chambers constructed near Athens, Georgia, USA

Treatment	July heat wave			August heat wave		
	PRE _{July}	DUR _{July}	POST _{July}	PRE _{August}	DUR _{August}	POST _{August}
$C_A T_{A0}$	34.7 (0.8)	37.0 (0.8)	36.9 (0.5)	35.3 (0.8)	36.8 (1.5)	35.0 (1.0)
$C_A T_{A3}$	37.3 (0.8)	39.8 (0.7)	39.2 (0.4)	37.4 (0.2)	38.8 (0.8)	37.6 (0.8)
$C_A T_{HW12}$	34.7 (0.8)	47.7 (1.0)	36.6 (0.5)	35.3 (0.6)	46.8 (0.9)	34.6 (1.0)
$C_E T_{A0}$	34.8 (0.7)	38.2 (0.4)	36.4 (0.6)	35.6 (0.4)	36.4 (0.5)	34.9 (0.9)
$C_E T_{A3}$	37.2 (0.8)	39.7 (0.7)	38.5 (0.4)	37.9 (0.1)	37.8 (0.9)	37.3 (0.9)
$C_E T_{HW12}$	34.7 (0.8)	47.4 (0.7)	36.1 (0.6)	35.5 (0.2)	47.0 (0.4)	34.9 (0.9)

Temperatures are averaged across measurement days before (PRE), during (DUR), and after (POST) applied heat waves in July and August 2010 at the time of measurement (12–14 h). Treatments were as follows: C_A , ambient [CO₂]; C_E , elevated [CO₂]; T_{A0} , ambient temperature; T_{A3} , ambient temperature +3 °C; T_{HW12} , 12 °C heat wave

treatment [CO₂] was 390 μmol mol⁻¹ for C_A and 715 μmol mol⁻¹ for C_E . Mean soil water content was 50 % v/v and did not significantly differ among [CO₂] × temperature treatments. Air temperatures were slightly higher (0.2–2 °C) during the July heat wave compared with the August heat wave (Table 1). Temperatures were equal between the [CO₂] treatments.

Leaf gas exchange and chlorophyll fluorescence parameters

Leaf gas exchange parameters were measured using a portable photosynthesis system (Model LI-6400XT, LiCor Biosciences, Lincoln, NE, USA) fitted with a fluorescence cuvette (6400-40 Leaf Chamber fluorometer, LiCor Biosciences, Lincoln, NE, USA). Light-saturated net photosynthesis (A_{net} , μmol CO₂ m⁻² s⁻¹), stomatal conductance (g_s , mol H₂O m⁻² s⁻¹), and transpiration rate (E , mmol H₂O m⁻² s⁻¹) were simultaneously measured. Intrinsic water use efficiency (WUE_i) was calculated per individual plant as the ratio of A_{net} to g_s . The temperature of the cuvette was set to match the temperature in the treatment chamber. The light source of the cuvette was maintained at 1,500 μmol PAR m⁻² s⁻¹ and [CO₂] was maintained at treatment conditions, either 380 or 700 μmol CO₂ mol⁻¹ for C_A and C_E , respectively. Relative humidity in the cuvette was maintained near 50 %. Leaf chlorophyll *a* fluorescence was measured immediately after gas exchange on the same leaves. Light-acclimated PSII efficiency ($F_v'/F_m' = 1 - F_0'/F_m'$), effective PSII quantum yield ($\Phi_{PSII} = 1 - F_s/F_m'$) and

photochemical quenching, which relates F_v'/F_m' to Φ_{PSII} and is a nonlinear measure for the proportion of open PSII centres ($q_P = [F_m' - F_s]/[F_m' - F_0']$), were calculated from steady-state fluorescence (F_s), maximum fluorescence after a saturating light flash (F_m' after 1 s 7,000 μmol m⁻² s⁻¹), and minimum fluorescence after a far-red pulse (F_0' after 3 s 9 μmol m⁻² s⁻¹ peaking at 740 nm). Measurements were performed on sunny or mostly sunny days. All measurements were conducted on foliage of the most recent fully developed flush.

Heat wave acclimation experiment

To determine whether seedlings were acclimating to repetitive exposure to +12 °C heat waves, three individuals from the T_{A0} treatment were placed in the T_{W12} treatment chamber right before the August heat wave (20–26 August 2010). Leaf gas exchange and chlorophyll *a* fluorescence measurements were conducted on these seedlings as well as on three seedlings that had been grown in the T_{HW12} treatment. These measurements took place 3 h before the consecutive heat waves experiment measurements (1200 and 1500 hours, respectively) on 19, 23 and 26, and 31 August (before, during, and after the +12 °C heat wave, respectively). This experiment allowed us to investigate whether seedlings with no previous exposure history (N-PEH) to a +12 °C heat wave performed similarly during a heat wave to those which were previously exposed (PEH). This factor was denoted as exposure history.

Consecutive heat waves experiment

Leaf gas exchange and chlorophyll *a* fluorescence measurements were conducted prior to, during, and after two midsummer heat wave treatment periods (20–26 July and 20–26 August, 2010, respectively). Measurements were conducted at noon on four randomly selected seedlings (different from the N-PEH and PEH seedlings) from each of the six treatment combinations. Different individuals in T_{HW12} treatment (i.e. PEH) than those used in the heat wave acclimation experiment were selected to avoid any artefacts caused by mechanical perturbation due to earlier handling of the seedlings. The rationale behind measuring the T_{HW12} seedlings again at this time is that photosynthetic activity can show a strong diurnal profile. Because of the 3 h difference between the two measurement sessions, it was decided that additional measurements in the T_{HW12} treatment were necessary. For the July heat wave treatment period, measurements were made over 10 days: 3 days before the start of the heat wave (13, 16, and 19 July), 4 days during the heat wave (20, 22, 24, and 26 July), and 3 days after the heat wave (27 and 29 July, and 1 August). For the August heat wave treatment period, measurements were made over 9 days: 2 days before the start of the heat wave (18 and 19 August), 4 days during the heat wave (20, 23, 24, and 26 August), and 3 days after the heat wave (27, 29, and 31 August).

Data analysis

For the acclimation experiment, leaf gas exchange and chlorophyll *a* fluorescence were analysed using a repeated-measures analysis of variance (RMANOVA) with $[CO_2]$ (2 levels; C_A , C_E) and previous +12 °C heat wave exposure history (2 levels; PEH, N-PEH) as the fixed effects, and treatment period (3 levels; PRE, DUR, POST) as the repeated effect. For the consecutive heat wave experiment, parameters were analysed using an RMANOVA with $[CO_2]$ (2 levels) and growth temperature (3 levels; T_{A0} , T_{A3} , T_{HW12}) as the fixed effects, and treatment period (3 levels) and heat wave number (2 levels; July, August) as the repeated effects. Tree ($n = 3$ or 4) was included as a random effect in all analyses. Student's *t* tests were used to compare between-treatment combinations of each parameter. Statistical tests were performed using PROC MIXED model analyses in SAS 9.4 (SAS

Institute Inc., Cary, NC, USA). The covariance structure with the lowest fit statistic (either -2 res log likelihood, AICc or BIC) was selected for the mixed model; all null model χ^2 tests yielded $P < 0.05$.

Results

Heat wave acclimation experiment

Previous exposure to +12 °C heat waves had a significant effect on A_{net} (Table 2). During all treatment periods, N-PEH seedlings had equal or higher A_{net} compared with PEH seedlings (+47 % across $[CO_2]$ treatments and periods; Fig. 2). Furthermore, A_{net} of N-PEH seedlings was 22 % lower during the heat wave than before, while A_{net} of PEH seedlings was 16 % higher during the heat wave than before (averaged across $[CO_2]$ treatments; both $P < 0.05$), indicated by an exposure history \times treatment period interaction.

Heat wave exposure history had no overall effect on g_s and E , but the interaction with treatment period was significant (Table 2). All PEH seedlings showed increased g_s and E during the heat wave, compared with values measured before the heat wave, and the effect was greatest in the C_E treatment (up to +307 % for g_s , $P < 0.001$; up to 375 % for E , $P < 0.001$; Figs. 2, 3). This increase did not occur in N-PEH seedlings, except for E in C_A .

There was a significant $[CO_2] \times$ history \times period effect on WUE_i . While all seedlings showed significantly decreased WUE_i during the heat wave (Fig. 3), this effect was most pronounced for PEH seedlings in the C_E treatment (−65 % compared with before the heat wave, $P < 0.001$). Lastly, photosynthetic efficiency parameters Φ_{PSII} and q_P were significantly affected by exposure history to +12 heat waves (Table 2). Averaged across $[CO_2]$ treatments and periods, Φ_{PSII} and q_P of N-PEH seedlings was 35 % and 33 % (respectively, both $P < 0.01$) higher than Φ_{PSII} and q_P of PEH seedlings (Fig. 4).

Consecutive heat waves experiment

In $C_A T_{HW12}$, compared to values before their respective heat waves (PRE_{July} and PRE_{August}), A_{net} showed a significant decline during the July heat wave (−61 %, $P < 0.001$) but not during the August heat

Table 2 Linear mixed model analysis of variance (*P* values) of effects on gas exchange and chlorophyll *a* fluorescence of 1-year-old northern red oak (*Quercus rubra* L.) seedlings grown under two CO₂ levels and with different heat wave exposure histories in August

Factor	Gas exchange				Fluorescence	
	<i>A</i> _{net}	<i>g</i> _s	<i>E</i>	WUE _i	Φ _{PSII}	<i>q</i> _P
<i>C</i>	0.016	0.253	0.229	< 0.001	0.618	0.361
<i>H</i>	0.044	0.095	0.092	0.502	0.009	0.003
<i>C</i> × <i>H</i>	0.836	0.286	0.311	0.766	0.597	0.655
<i>P</i>	0.093	< 0.001	< 0.001	< 0.001	< 0.001	0.107
<i>C</i> × <i>P</i>	0.058	0.058	0.021	< 0.001	0.148	0.449
<i>H</i> × <i>P</i>	0.032	0.022	0.004	0.026	0.057	0.097
<i>C</i> × <i>H</i> × <i>P</i>	0.103	0.108	0.019	0.031	0.065	0.684

Factors are [CO₂] (*C*; 2 levels: 380 and 700 μmol CO₂ mol⁻¹), heat wave exposure history (*H*; 2 levels: no previous exposure, and three previous +12 °C heat waves). Values < 0.05 denote significance of the effect and are presented in bold font

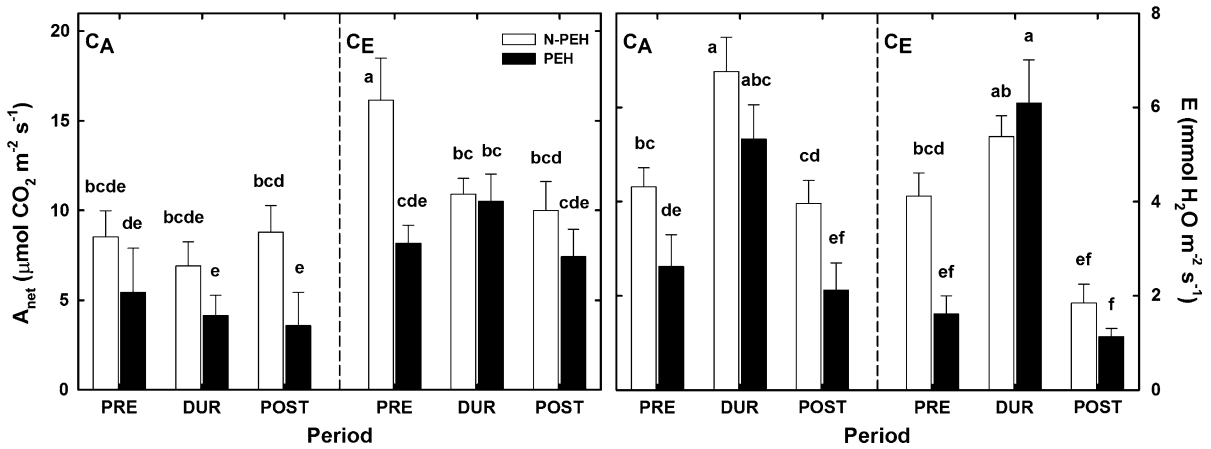


Fig. 2 Mean (±SE, *n* = 3) net photosynthesis (*A*_{net}) and transpiration (*E*) of *Q. rubra* seedlings grown under two [CO₂] treatments: 380 (*C*_A) and 700 (*C*_E) μmol CO₂ mol⁻¹, and two temperature treatments: ambient (*T*_{A0}), and +12 °C heat wave (*T*_{HW12}). Before the start of the August heat wave, the *T*_{A0} seedlings were placed in the *T*_{HW12} chamber to act as

controls in order to test putative acclimation of the *T*_{HW12} seedlings to previous heat wave exposure. *T*_{A0} seedlings were hence denoted as having no previous exposure history (N-PEH), while *T*_{HW12} seedlings were denoted as having previous exposure history (PEH). Different lowercase indices (*a*, *b*, ...) indicate significant differences between all values at *P* = 0.05

wave (−19 %, *P* = 0.26). In contrast, in the *C*_E*T*_{HW12} treatment, significant declines in *A*_{net} were found in during both heat waves (−40 %, *P* < 0.001 during the July heat wave and −49 %, *P* < 0.001 during the August heat wave) (Fig. 5).

There was no significant temperature effect on *g*_s during the July heat wave (Table 3). In contrast, across [CO₂] treatments and treatment periods during the August heat wave, there was a significant reduction in *g*_s in *T*_{HW12} compared with *T*_{A0} (−30 %, *P* < 0.05). In addition, there was a significant temperature × treatment period interaction during the August heat wave, in which *g*_s in the different temperature treatments

evolved differently over the course of the three periods. Notably, *g*_s of the *T*_{HW12} seedlings increased during the August heat wave in both *C*_A and *C*_E and dropped again post-heat wave. This behaviour was not observed during the July heat wave. Seedlings in *C*_A*T*_{HW12} showed no significant difference in *g*_s during the July heat wave compared with pre-heat wave, but showed a significant decline post-heat wave. Seedlings in *C*_E*T*_{A0} had higher *g*_s during the July heat wave (+27 %, *P* < 0.05) (Fig. 6).

A significant treatment period effect and treatment period × temperature interaction was present for *E* in both heat waves (Table 3). During the July heat wave,

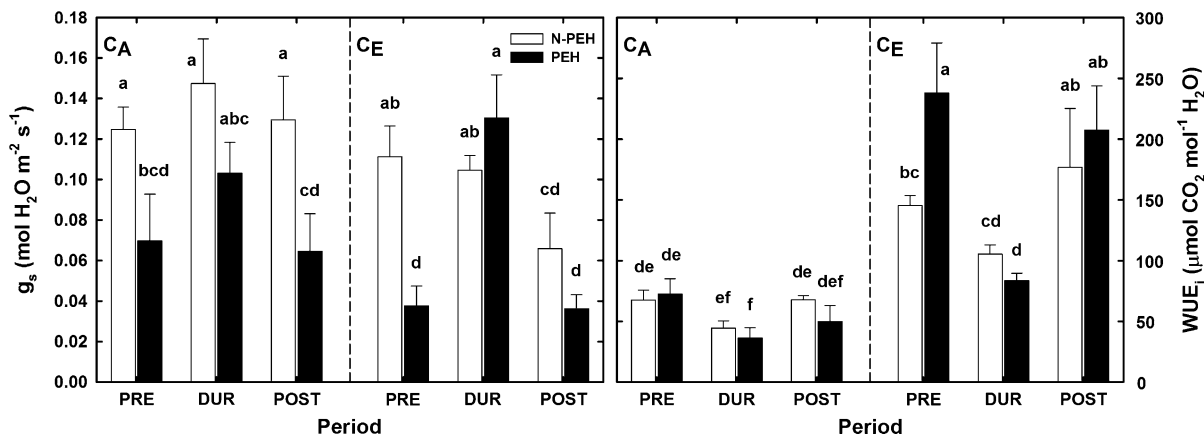


Fig. 3 Mean (\pm SE, $n = 3$) stomatal conductance (g_s) and intrinsic water use efficiency (WUE_i) of *Q. rubra* seedlings grown under two $[CO_2]$ treatments: 380 (C_A) and 700 (C_E) $\mu\text{mol CO}_2 \text{ mol}^{-1}$, and four temperature treatments: ambient (T_{A0}), and

+12 °C heat wave (T_{HW12}). N-PEH: seedlings with no previous heat wave exposure history; PEH: seedlings with previous heat wave exposure history. Different lowercase indices (a, b, \dots) indicate significant differences between all values at $P = 0.05$

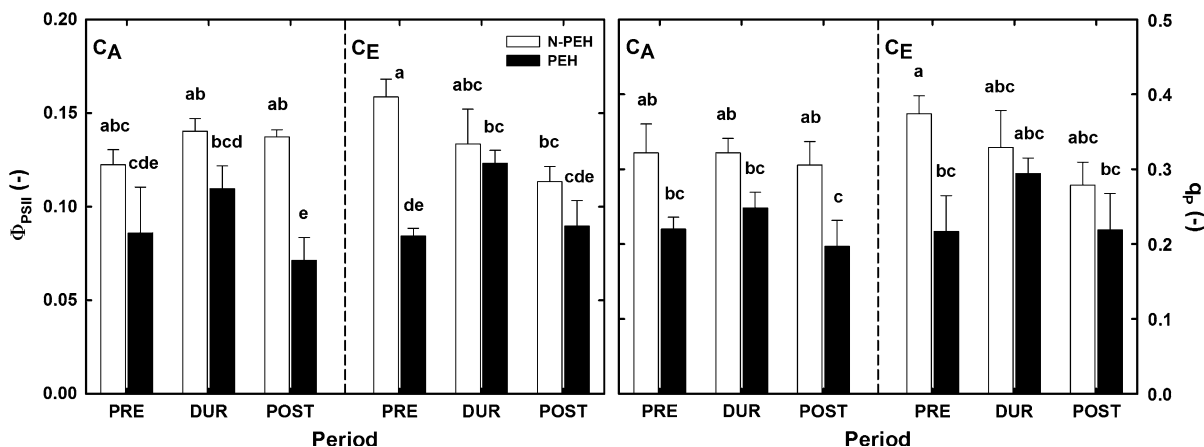


Fig. 4 Mean (\pm SE, $n = 3$) effective PSII quantum yield (Φ_{PSII}) and photochemical quenching (q_p) of *Q. rubra* seedlings grown under two $[CO_2]$ treatments: 380 (C_A) and 700 (C_E) $\mu\text{mol CO}_2 \text{ mol}^{-1}$, and four temperature treatments: ambient (T_{A0}), and

+12 °C heat wave (T_{HW12}). N-PEH: seedlings with no previous heat wave exposure history; PEH: seedlings with previous heat wave exposure history. Different lowercase indices (a, b, \dots) indicate significant differences between all values at $P = 0.05$

E significantly increased in all treatments, with the exception of $C_E T_{A3}$, where the increase was not significant. The strongest increase was found in the T_{HW12} seedlings. Accordingly, during the August heat wave, the highest E was also found in T_{HW12} seedlings (Fig. 6).

During the July heat wave, no significant differences were found for Φ_{PSII} (Table 3), although seedlings in $C_A T_{HW12}$ showed a reduction in Φ_{PSII} of -22% ($P < 0.05$) compared with PRE_{July} and did not recover during the 5 days after the heat wave (-27% , $P < 0.05$). During the August measurements, significant $[CO_2]$, temperature and treatment period effects were found (Table 3). Here, seedlings in $C_E T_{HW12}$

showed a smaller reduction in Φ_{PSII} (-22% , $P < 0.01$) than $C_A T_{HW12}$ (-29% , $P = 0.08$), which persisted after the heat wave (-17% , $P < 0.05$), though values were still higher ($+149\%$, $P < 0.001$) in $C_E T_{HW12}$ than in $C_A T_{HW12}$ (Fig. 7).

A significant $[CO_2] \times$ temperature interaction affected F_v'/F_m' during the July measurements. Averaged across the treatment period, no significant differences were found between the temperature treatments at ambient $[CO_2]$. However, F_v'/F_m' was significantly higher in $C_E T_{HW12}$ compared with $C_E T_{A0}$ ($+17\%$, $P < 0.05$). During the August measurements, there was a significant treatment period \times temperature interaction on F_v'/F_m' (Table 3).

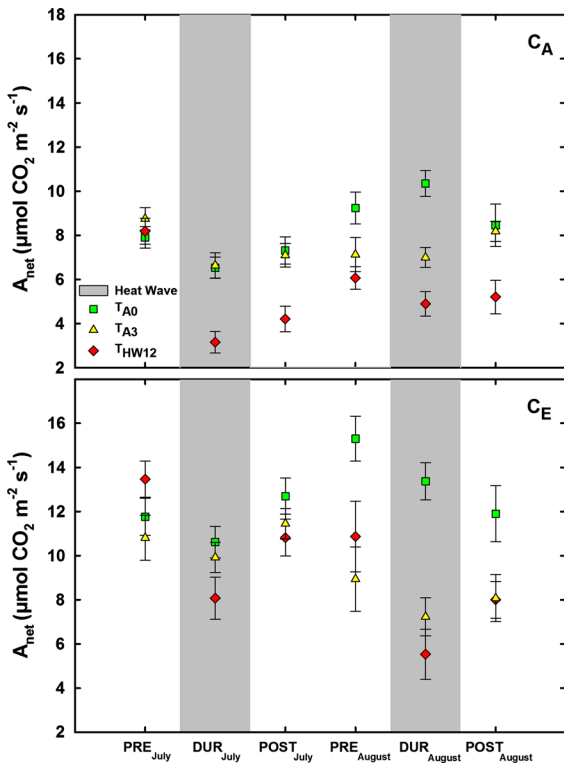


Fig. 5 Mean (\pm SE, $n = 4$) net photosynthesis (A_{net}) of *Q. rubra* seedlings grown under two $[\text{CO}_2]$ treatments: 380 (C_A) and 700 (C_E) $\mu\text{mol CO}_2 \text{ mol}^{-1}$, and three temperature treatments: ambient (T_{A0}), ambient +3 °C (T_{A3}), and +12 °C heat wave ($T_{\text{HW}12}$). Measurements were performed before (PRE), during (DUR), and after (POST) two heat waves in July and August 2010

Compared with pre-heat wave values, F_v'/F_m' in $C_A T_{A0}$ and $C_A T_{A3}$ was significantly higher during the August heat wave, while $C_A T_{\text{HW}12}$ and $C_E T_{\text{HW}12}$ declined significantly

Table 3 Linear mixed model analysis of variance (P values) of effects on gas exchange and chlorophyll a fluorescence of 1-year-old northern red oak (*Quercus rubra* L.) seedlings grown under two CO_2 levels and three temperature treatments during July and August

Effect	July heat wave					August heat wave				
	A_{net}	g_s	E	F_v'/F_m'	Φ_{PSII}	A_{net}	g_s	E	F_v'/F_m'	Φ_{PSII}
C	<0.001	0.001	0.003	0.001	0.924	0.016	<0.001	0.003	0.025	<0.001
T	0.192	0.970	0.087	0.179	0.118	0.002	0.029	0.087	0.071	0.009
$C \times T$	0.228	0.266	0.338	0.021	0.748	0.331	0.400	0.338	0.737	0.074
P	<0.001	0.018	<0.001	0.919	0.399	0.004	0.007	<0.001	0.058	0.005
$C \times P$	0.079	0.217	0.590	0.089	0.254	0.007	0.684	0.590	0.034	0.104
$T \times P$	<0.001	0.096	<0.001	0.122	0.327	0.005	0.049	<0.001	<0.001	0.065
$T \times C \times P$	0.959	0.904	0.863	0.784	0.574	0.649	0.957	0.863	0.412	0.957

Factors are $[\text{CO}_2]$ (C ; 2 levels: 380 and 700 $\mu\text{mol CO}_2 \text{ mol}^{-1}$), growth temperature (T ; 3 levels: ambient, ambient +3 °C, and ambient +12 °C every fourth week), period (P ; 3 levels: before, during and after the heat wave). Values < 0.05 denote significance of the effect and are presented in bold font

(−29 %, $P < 0.001$ and −33 %, $P < 0.001$; respectively). After the heat wave, $C_A T_{\text{HW}12}$ recovered completely while $C_E T_{\text{HW}12}$ was still significantly lower (−13 %, $P < 0.05$) compared with pre-heat wave values (Fig. 7).

During both July and August, there was a significant effect of elevated $[\text{CO}_2]$ on A_{net} , g_s , and E (Table 3). Averaged across all temperature treatments and periods within a monthly measurement, A_{net} was 67 % ($P < 0.001$) and 30 % ($P < 0.01$) higher in the elevated $[\text{CO}_2]$ treatment compared to ambient $[\text{CO}_2]$ in July and August, respectively. A stronger effect of elevated $[\text{CO}_2]$ on g_s was found in August (−50 %, $P < 0.001$) than in July (−26 %, $P < 0.01$). A stronger $[\text{CO}_2]$ effect on E was also observed in August (−45 %, $P < 0.001$) compared with July (−22 %, $P < 0.01$). Elevated $[\text{CO}_2]$ had a significant effect on F_v'/F_m' across all treatment periods, but only in August on Φ_{PSII} (Table 3). F_v'/F_m' was significantly higher in elevated $[\text{CO}_2]$ in both months (July: +14 %, $P < 0.01$; August: +11 %, $P < 0.05$). While there was no effect of elevated $[\text{CO}_2]$ on Φ_{PSII} in July heat wave, it was significantly higher (+144 %, $P < 0.001$) in August compared with ambient $[\text{CO}_2]$.

Discussion

Acclimation after repeated heat waves at monthly intervals

Our first hypothesis was that seedlings with a history of heat wave exposure (PEH seedlings) would have higher

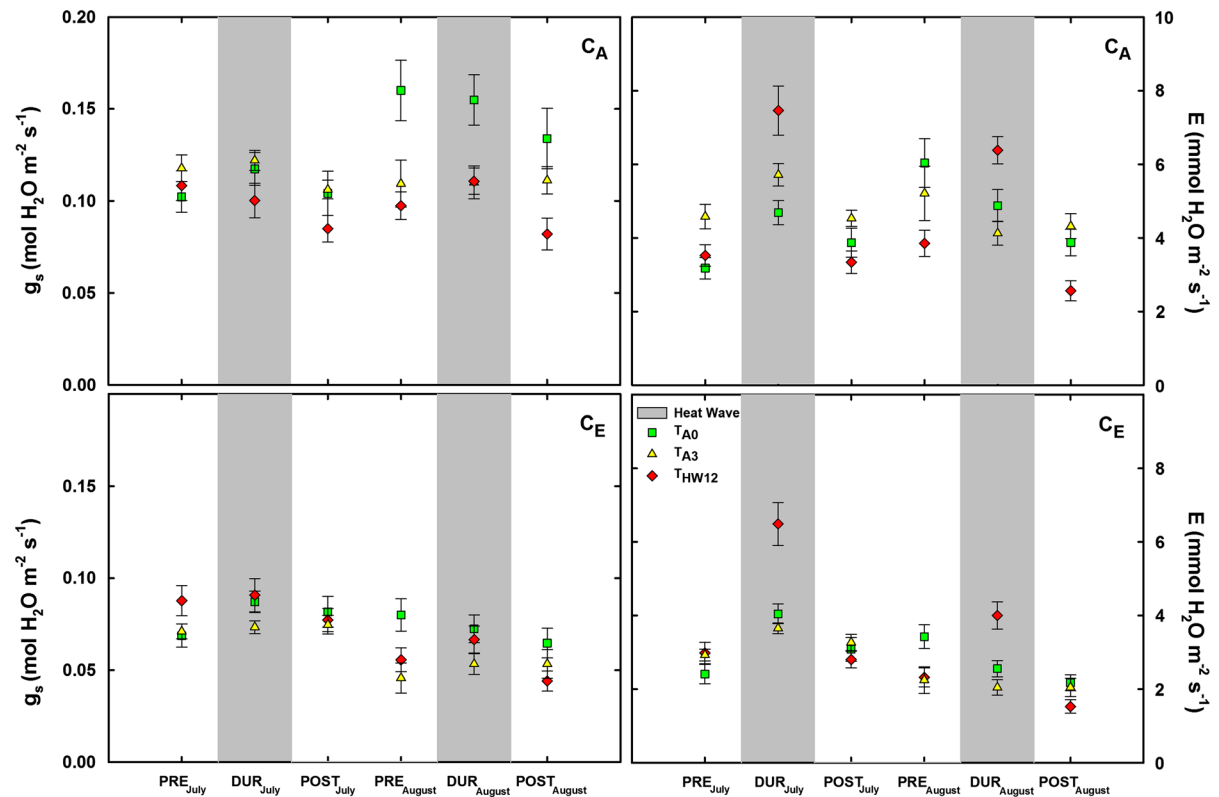


Fig. 6 Mean (\pm SE, $n = 4$) stomatal conductance (g_s) and transpiration (E) of *Q. rubra* seedlings grown under two [CO_2] treatments: 380 (C_A) and 700 (C_E) $\mu\text{mol CO}_2 \text{ mol}^{-1}$, and three temperature treatments: ambient (T_{A0}), ambient +3 °C (T_{A3}),

and +12 °C heat wave (T_{HW12}). Measurements were performed before (PRE), during (DUR), and after (POST) two heat waves in July and August 2010

net photosynthesis (A_{net}) and effective PSII quantum yield (Φ_{PSII}) than seedlings with no history of heat wave exposure (N-PEH seedlings), when exposed to a +12 °C heat wave. This hypothesis was not supported by our results, as average A_{net} and Φ_{PSII} were equal or higher for the N-PEH seedlings than for the PEH seedlings during all treatment periods. Part of this difference in A_{net} response may have been due to the significantly lower stomatal conductance (g_s) of the PEH seedlings during all but one treatment period, as stomatal closure may limit diffusion of atmospheric CO_2 into the leaf, leading to reduced carboxylase activity (Farquhar et al. 1980). In a successive heat wave experiment on arctic species, Marchand et al. (2006) also found that g_s of individuals previously exposed to heat waves was lower outside the heat waves than g_s of control individuals. Higher g_s of N-PEH seedlings compared with PEH seedlings during the heat wave might be explained by a greater need for the N-PEH seedlings to evaporatively cool their leaf tissue. Plants with a history of heat exposure often apply

biochemical methods to deal with an additional heat event (Vierling 1991) and are able to withstand higher ambient temperatures without increasing g_s to cool evaporatively (Fischer et al. 1970). At 35 °C (PRE_{August}), PEH seedlings therefore might not have been experiencing heat stress, while N-PEH seedlings were. In addition to being higher, g_s of N-PEH seedlings lacked some or all of the dynamic behaviour of g_s observed in the PEH seedlings. We suggest that the dynamic stomatal behaviour of the PEH seedlings reflects acclimation of water strategy in response to the T_{HW12} treatment. Reduced g_s during periods of relatively low temperatures allowed these seedlings to reduce water loss and store water, perhaps against potential high temperatures in the future when soil water might be more limited, a response that prioritises survival over carbon gain.

Our second hypothesis, that elevated [CO_2] would mitigate differences in A_{net} and Φ_{PSII} between N-PEH and PEH seedlings, was supported during and after the heat wave. Previous studies have shown that stimulation

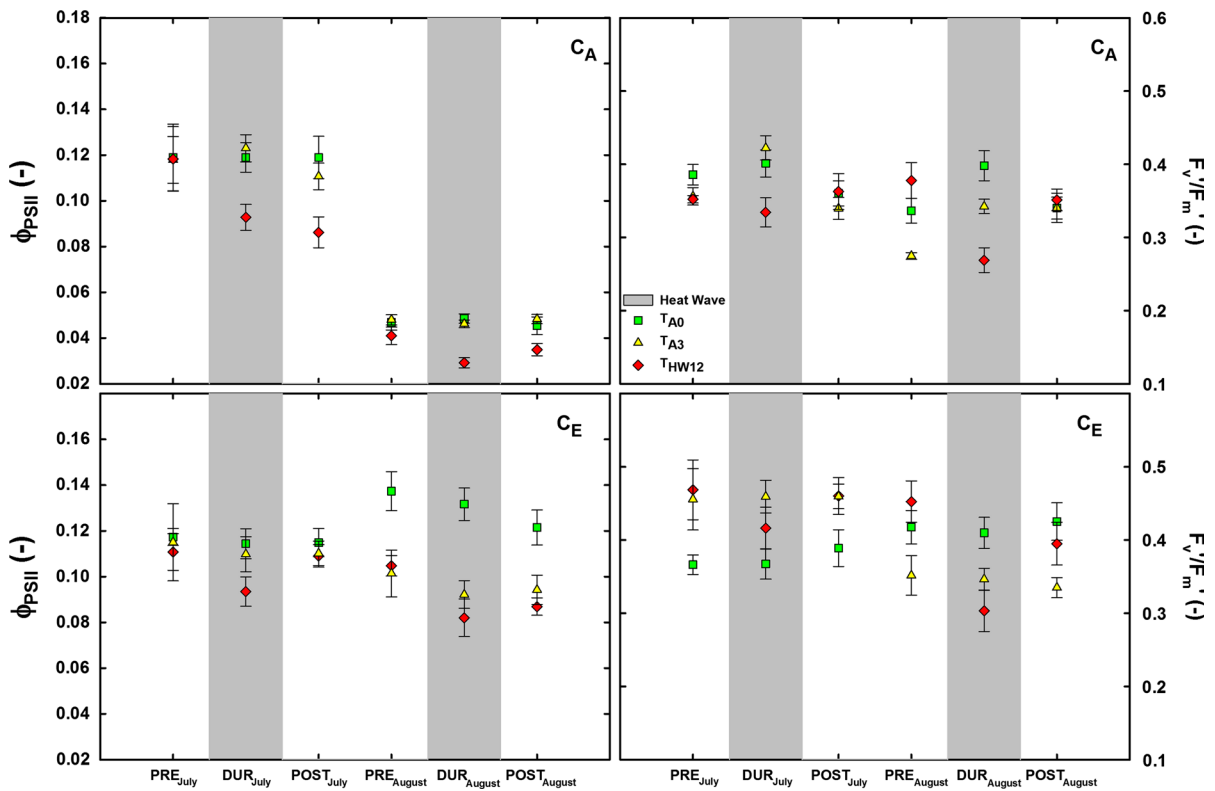


Fig. 7 Mean (\pm SE, $n = 4$) effective PSII quantum yield (Φ_{PSII}) and light-acclimated PSII efficiency (F_v'/F_m') of *Q. rubra* seedlings grown under two $[CO_2]$ treatments: 380 (C_A) and 700 (C_E) $\mu\text{mol } CO_2 \text{ mol}^{-1}$, and three temperature

treatments: ambient (T_{A0}), ambient +3 °C (T_{A3}), and +12 °C heat wave (T_{HW12}). Measurements were performed before (PRE), during (DUR), and after (POST) two heat waves in July and August 2010

of photosynthesis by elevated $[CO_2]$ increases under higher temperatures (Sage and Kubien 2007), which could explain our results during the heat wave. The reason for the dissimilar response between our results before and after the heat wave is less clear, but might mean that the PEH seedlings suffered lasting damage during the July heat wave that reduced A_{net} . It would furthermore seem that A_{net} and Φ_{PSII} of N-PEH seedlings did not recover to their PRE values in the C_E treatment, while they did in the C_A treatment. It is possible that the concurrent decrease in g_s of the C_E N-PEH seedlings impeded evaporative cooling, and therefore contributed to this reduced photosynthetic activity.

Photosynthetic responses to consecutive heat waves

The severe heat wave had a significant impact on A_{net} during July in both $[CO_2]$ treatments. However, this

response was less distinct during the August heat wave in the C_A treatment, which could suggest thermal acclimation to the very high July heat wave temperature (Sage and Kubien 2007; Yamori et al. 2014). This finding further supports our third hypothesis that A_{net} differences between T_{A0} and T_{W12} would be smaller during the August heat wave than during the July heat wave, possibly because of photosynthetic acclimation. Thermal acclimation to higher temperatures in tree species has previously been reported in *Pinus taeda* (Teskey and Will 1999), *Quercus* species (Gunderson et al. 2010) and three tropical tree species (Cheesman and Winter 2013). However, because a constant moderate temperature increase was applied in these studies rather than a severe heat stress event, we suggest that a shift in temperature optimum does not completely explain the smaller decrease in A_{net} after the August heat wave. Surprisingly, no increased thermotolerance of A_{net} was found under elevated

[CO₂] in the T_{HW12} treatment. On the contrary, the August heat wave seemed to have had a greater negative effect on A_{net} of the T_{HW12} seedlings under elevated [CO₂] than under ambient [CO₂]. It is possible that the reduced A_{net} of the $C_E T_{HW12}$ seedlings was caused by the lower g_s values during the August heat wave.

Acclimation to high temperatures has also been associated with acclimation of Rubisco activase activity, expression of heat-shock proteins, and thermal acclimation of respiration (Yamori et al. 2014). At ambient [CO₂], the reduction in A_{net} coincided with decreased Φ_{PSII} during and after both heat waves in the T_{HW12} treatments, and also coincided with decreased light-acclimated PSII efficiency (F_v'/F_m') during the August heat wave. Counter-intuitively, at elevated [CO₂] both F_v'/F_m' and Φ_{PSII} exhibited an equal or more severe decline in the August heat wave compared with the July heat wave. As suggested in Ameye et al. (2012), the decline and partial recovery of Φ_{PSII} can be associated with reversible thermal deactivation of Rubisco activase (Haldimann and Feller 2004). However, no signs of acclimation in fluorescence parameters were found during the second (August) heat wave and acclimation of Rubisco activase would result in only small changes in A_{net} , suggesting that these were not the underlying mechanisms for thermal acclimation of A_{net} (Cavanagh and Kubien 2014).

A number of studies have reported that an increase in temperature had a positive effect on photosynthetic performance and growth (Sage and Kubien 2007; Saxe et al. 2001; Way and Oren 2010). We observed a number of negative effects of short-term extreme heat events on gas exchange physiology, including reduced A_{net} , Φ_{PSII} , and F_v'/F_m' . Filewod and Thomas (2014) showed that 3 days of record high temperatures (31–33 °C) in May had a lasting impact on photosynthetic potential of *Acer saccharum* during the rest of the growing season. Besides effects on photosynthesis, plant reproduction may become impaired. Abeli et al. (2012) observed that, following the 2003 heat wave in Europe, flower production in *Alopecurus alpinus* and *Vicia cusnae* was significantly decreased compared with previous years.

It has already been reported for *Arabidopsis* that pre-exposure to moderate heat stress resulted in a higher accumulation of different molecular chaperones such as small heat-shock proteins and HSP70s, as well as reactive oxygen species and redox response

enzymes, such as ascorbate peroxidase, which resulted in a higher survival rate after a more severe heat stress treatment (Mittler et al. 2012). In addition, inducible photoprotective mechanisms were shown for different Mediterranean and Atlantic species after the European heat wave of 2003, with the accumulation of the antioxidant tocopherol and xanthophyll cycle pigments (Garcia-Plazaola et al. 2008) which eventually led to increased thermotolerance (Zsofi et al. 2009). The observed decrease in F_v'/F_m' during the heat waves in this experiment may therefore be an indication of greater photoprotection through increased activation of the xanthophyll cycle.

The rise in A_{net} in response to long-term exposure to elevated [CO₂] observed in this study is consistent with the literature. Such positive effects have previously been demonstrated for *Quercus rubra* (Anderson and Tomlinson 1998; Cavender-Bares et al. 2000; Kubiske and Pregitzer 1996). Growth at elevated [CO₂] has been shown to mitigate heat stress effects on A_{net} (Sage and Kubien 2007), and such mitigation was found during the July heat wave (Ameys et al. 2012), but was absent during the August heat wave (Fig. 4), thus partly confirming our fourth hypothesis that elevated [CO₂] would mitigate differences in A_{net} and Φ_{PSII} among the temperature treatments during both heat waves. Similar results have also been reported by Hamilton et al. (2008), who investigated the interactive effects of temperature and [CO₂] on pre-heat-stressed plants. They found for the C3 species *Chenopodium album* and *Pisum sativum* that elevated [CO₂] mitigated the heat (+15 °C) stress effects on photosynthesis of plants with no previous +15 °C exposure. However, plants pre-exposed to temperatures 15 °C above growth temperature did not experience any mitigating effect. It is notable that the A_{net} response to a +12 °C August heat wave is different in Fig. 1 than in Fig. 4. The likely explanation lies in the difference in measurement time between the two experiments. The acclimation experiment measurements (Fig. 1) were performed around 1200 hours, while the consecutive heat wave experiment measurements (Fig. 4) were performed around 1500 hours. It is known that gas exchange and chlorophyll *a* fluorescence values can show a strong diurnal profile, with photosynthetic activity generally being highest in the morning. We, therefore, argue that at 1200 hours, temperatures were not yet high enough to elicit the same response as they did at 1500 hours (Fig. 1b shows the average diurnal temperature profile).

Stomatal responses to successive heat waves

We found no temperature effect on g_s in July, suggesting that the seedlings did not close their stomata to preserve water content in leaves, which was dissimilar to what we saw in August. Since these seedlings had access to an adequate water supply, this apparent lack of response may reflect a strategy of the plants to evaporatively cool their leaves through transpiration. This idea is largely supported by the fact that transpiration rose to values between +66 and +112 % in the plants exposed to the July and August heat waves. The absence of a temperature effect on g_s was also reported for two *Eucalyptus* species, which were subjected to a +4 °C treatment, and were watered daily (Ghannoum et al. 2010).

In conclusion, we observed that severe heat waves reduced A_{net} in both July and August compared to pre-heat wave values, and that average A_{net} was significantly lower in the heat wave treatment than in the ambient temperature treatments during the heat wave periods. Our results suggest that *Q. rubra* seedlings subjected to consecutive heat waves may moderate stomatal conductance outside the heat wave as a means to conserve water at lower temperatures. Thus, following an extreme and lengthy heat wave, a decrease in forest productivity, such as the one reported after the 2003 European heat wave (Ciais et al. 2005), may reflect a mechanism to conserve water that prioritizes survival over carbon acquisition. Since heat waves will have a different impact on photosynthetic performance than long-term smaller changes in average temperature, a better understanding of photosynthetic performance both during and after heat waves is of paramount importance if we hope to better understand how plants will respond to changes in climate.

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