The effect of heat waves, elevated [CO$_2$] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings

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Abstract

The frequency and intensity of heat waves are predicted to increase. This study investigates whether heat waves would have the same impact as a constant increase in temperature with the same heat sum, and whether there would be any interactive effects of elevated [CO$_2$] and soil moisture content. We grew *Quercus rubra* seedlings in treatment chambers maintained at either ambient or elevated [CO$_2$] (380 or 700 μmol CO$_2$ mol^{-1}) with temperature treatments of ambient, ambient +3 °C, moderate heat wave (+6 °C every other week) or severe heat wave (+12 °C every fourth week) temperatures. Averaged over a 4-week period, and the entire growing season, the three elevated temperature treatments had the same average temperature and heat sum. Half the seedlings were watered to a soil water content near field capacity, half to about 50% of this value. Foliar gas exchange measurements were performed morning and afternoon (9:00 and 15:00 hours) before, during and after an applied heat wave in August 2010. Biomass accumulation was measured after five heat wave cycles. Under ambient [CO$_2$] and well-watered conditions, biomass accumulation was highest in the +3 °C treatment, intermediate in the +6 °C heat wave and lowest in the +12 °C heat wave treatment. This response was mitigated by elevated [CO$_2$]. Low soil moisture significantly decreased net photosynthesis (A_{net}) and biomass in all [CO$_2$] and temperature treatments. The +12 °C heat wave reduced afternoon A_{net} by 23% in ambient [CO$_2$]. Although this reduction was relatively greater under elevated [CO$_2$], A_{net} values during this heat wave were still 34% higher than under ambient [CO$_2$]. We concluded that heat waves affected biomass growth differently than the same amount of heat applied uniformly over the growing season, and that the plant response to heat waves also depends on [CO$_2$] and soil moisture conditions.

Keywords: climate extreme, drought, elevated [CO$_2$], global warming, growth inhibition, heat wave, photosynthesis, *Quercus rubra*

Introduction

The main constituents of global climate change are generally considered to be an increase in atmospheric carbon dioxide concentration ([CO$_2$]) and other greenhouse gases (Whorf & Keeling, 1998; IPCC, 2007a), an increase in average temperature (Houghton *et al.*, 2001), an increase in the frequency of climate extremes including heat waves (Meehl & Tebaldi, 2004; Schar *et al.*, 2004; Della-Marta *et al.*, 2007; Lynn *et al.*, 2007), a change in average precipitation (Roy *et al.*, 2012) and an increase in precipitation variability (Dore, 2005; Meehl *et al.*, 2007; Trenberth *et al.*, 2007).

From 1949 to 1995, the number of summer heat waves in the eastern United States increased by 20% (Gaffen & Ross, 1998). Similarly, summer heat waves have doubled in length (from 1.4 to 3.0 days per heat wave), and the frequency of hot days tripled in Western Europe in recent years compared with the early 20th century (Della-Marta *et al.*, 2007). This trend is considered very likely to continue in this century (IPCC, 2007b; Lynn *et al.*, 2007). Such extreme heat events may be more important to many organisms than shifts in average temperature (Easterling *et al.*, 2000) and have the potential to trigger threshold-type responses in plants and ecosystems that might prevent acclimation or recovery upon return to normal environmental conditions (Bragazza, 2008; Jentsch *et al.*, 2011). The intensity of the extreme is expected to be a determining factor in this context (Marchand *et al.*, 2006; Hüve *et al.*, 2011). Heat waves likely have more significant negative impacts on plant communities and ecosystems than the same heat sum received over longer periods of time

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(Pichler & Oberhuber, 2007). A 30% decrease in gross primary productivity in 2003 in forests across Western Europe was attributed to a severe heat wave that summer (Ciais *et al.*, 2005).

Productivity decreases were further amplified in regions within Europe that were impacted by drought, a factor usually accompanying heat waves (De Boeck *et al.*, 2010; De Boeck *et al.*, 2011). Other reported stress influences include metabolite accumulation, leaf senescence and a decrease in photosynthetic efficiency, stomatal conductance and protein synthesis (Ohashi *et al.*, 2006; Dai *et al.*, 2007; Warren *et al.*, 2011). The simultaneous occurrence of these extreme conditions emphasizes the need for investigation of their impact (Fensham & Holman, 1999; Adams *et al.*, 2009; Allen *et al.*, 2010).

The effect of elevated temperatures on plant physiological processes and growth is often evaluated by comparing different constant temperatures (e.g. Roden & Ball, 1996; Qaderi *et al.*, 2006; Ow *et al.*, 2008) or comparing a constant elevation in temperature to ambient conditions (e.g. Berry & Björkman, 1980; Gunderson *et al.*, 2009; Dias *et al.*, 2011; Djanaguiraman *et al.*, 2011; Wertin *et al.*, 2011). Way & Oren (2010) concluded in a review of such studies that elevated temperatures enhanced growth in most tree species. However, it is unlikely that air temperature will increase gradually and evenly. Instead, the frequency and intensity of heat waves are predicted to increase (Breshears *et al.*, 2005; Diffenbaugh & Ashfaq, 2010).

Elevated $[CO_2]$ has been reported to have had a mitigating effect on heat stress in short-term studies lasting less than 1 day (Faria *et al.*, 1998; Huxman *et al.*, 1998; Hüve *et al.*, 2011) and in one instance, 1 week (Hamerlynck *et al.*, 2000). Leaves from woody and herbaceous plants grown in elevated $[CO_2]$ maintained higher A_{net} when exposed to heat stress than leaves grown at ambient $[CO_2]$ (Taub *et al.*, 2000). Elevated $[CO_2]$ was also reported to enhance thermotolerance of A_{net} in a group of C3 plants, allowing the plants to photosynthesize at higher rates for a longer duration of time, during a 4 h exposure to 40 °C (Wang *et al.*, 2008). However, in some cases, a negative interactive effect was found between elevated $[CO_2]$ and temperature (Andre & du Cloux, 1993; Prasad *et al.*, 2006; Hatfield *et al.*, 2011).

These and other studies have shown that plant responses to simultaneously occurring climate change factors (elevated $[CO_2]$, increased temperature, drought) are, in all likelihood, not simply predictable as additive responses of the individual factors (Shaw *et al.*, 2002; Norby & Luo, 2004). For example, under elevated $[CO_2]$, drought or temperature stress resistance may increase through improved carbon and water status (Centritto *et al.*, 1999; Luis *et al.*, 1999; Medlyn *et al.*, 2001; Gunderson *et al.*, 2002; Albert *et al.*, 2011; Naudts *et al.*, 2011). Resistance to both heat and drought stress under elevated $[CO_2]$ has been studied and observed as well, although to a lesser extent (e.g. Caldwell *et al.*, 2005). To date, experiments on the impact of extreme heat events on plants for more than a few hours are scarce (Hamerlynck *et al.*, 2000) and to our knowledge, there have been no studies on the interactive effect of elevated $[CO_2]$ and drought on plant performance during heat waves of different frequency and intensity. The objective of this study was to examine the effect of heat waves of different frequency and intensity on foliar gas exchange and biomass growth of northern red oak seedlings and to determine whether the effects are modified by elevated $[CO_2]$ or decreased soil water availability. Our specific hypotheses were as follows: (1) the most intense heat wave treatment will have the most negative effect on gas exchange and growth; (2) decreased soil moisture will exacerbate the heat wave impact; (3) elevated $[CO_2]$ will mitigate the heat wave impact and (4) heat waves will affect growth more negatively than the same cumulative heat exposure produced by a constant increase in temperature.

Materials and methods

Study site

The study site was located at Whitehall Forest of the University of Georgia in Athens (33°57′N, 83°19′W, altitude 230 m). Eight 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 treatment chambers were constructed according to the method described in Boyette & 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 minimize shading and oriented facing south to maximize daily sun exposure.

Plant material

Seeds of northern red oak (*Q. rubra* L.) were planted 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, Lecompte, LA, USA). The provenance is from the southernmost region of the species range (Little, 1971).

In February 2010, the seedlings were transplanted to 8 L pots in the same potting medium. The seedlings were watered three times a day until saturation. In April, May, June and July, each pot was fertilized with 30 g of 15-9-12 extended release fertilizer (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 control pests (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 chamber was assigned one of eight treatment combinations. These combinations were produced by two factors namely atmospheric $[CO_2]$ and chamber temperature. The $[CO_2]$ treatments were ambient $[CO_2]$ (C_A, 380 μmol CO_2 mol^{-1}) or elevated $[CO_2]$ (C_E, 700 μmol CO_2 mol^{-1}). The temperature treatments were ambient (T_{A0}), ambient +3 °C (T_{A3}), one heat wave elevating ambient by 6 °C every other week (T_{W6}) and one heat wave elevating ambient by 12 °C every 4 weeks (T_{W12}). The ambient temperature was measured outside the chambers. Within each chamber, we assigned half of the seedlings to one of two water treatments. These water treatments consisted of watering to maintain target volumetric water content of 50% (W_H) or 30% (W_L). Thus, the experiment was a complete factorial of two $[CO_2]$ × four temperature × two water treatments.

Thirty northern red oak seedlings were placed in each chamber. The seedlings were randomly assigned to one of six blocks per chamber. Three blocks were assigned to the W_H treatment and three to the W_L treatment. Pots were evenly spaced within the chamber. To minimize any potential chamber effect, each month the treatment combinations, blocks and pots within blocks were randomly rotated among the chambers, and chamber CO_2 and temperature conditions were adjusted accordingly. In addition, as described in more detail below, environmental conditions were monitored continuously in each chamber to ensure that the $[CO_2]$ and soil moisture treatments were statistically similar among treatment levels in all chambers and that the air temperature treatments met their target values. Treatments were initiated 2 May 2010. In this study, we obtained gas exchange data before, during and after the fourth heat wave applied from 20 to 26 August 2010, i.e. during the peak of the growing season.

In this study, we simulated the effects of heat waves in a way that could be compared to many previous studies in which air temperature was constantly elevated by a fixed amount. For this study, we have two controls, T_{A0} and T_{A3}, i.e. ambient temperature and ambient +3 °C. The +6 °C heat wave treatment (T_{W6}) was held at ambient temperature for 1 week and subjected to ambient +6 °C the following week. The +12 °C heat wave treatment (T_{W12}) was held at ambient temperature for 3 weeks and subjected to ambient +12 °C every fourth week (treatment conditions for August 2010 are shown in Fig. 1a and b). Both heat wave treatment schemes were repeated every 4 weeks throughout the growing season. The elevated temperature treatments T_{A3}, T_{W6} and T_{W12} represent the same applied heat sum and correspond to an average increase of 3 °C over a 4-week period and the entire growing season.

To maintain $[CO_2]$ at the desired levels, a nondispersive infrared CO_2-sensor (model GMT222; Vaisala Inc., Woburn, MA, USA) continuously measured $[CO_2]$ in 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. Chamber

temperature was measured by type T thermocouples every 3 min and averaged and recorded every 15 min using a datalogger (Campbell 23X, Logan, UT, USA), which continuously compared the air temperature inside each chamber to the outside temperature measured with matched thermocouples located 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 dataloggers controlled an air conditioner (model FAM186R2A; Frigidaire, Augusta, GA, USA) and a heater (model 3VU33A; Dayton Electric, Niles, IL, USA) in each chamber, to maintain the treatment air temperatures.

Soil water content in the pots was measured by ECH2O EC5 soil moisture probes (Decagon Devices, Pullman, WA, USA) and recorded every 10 min. Probes were inserted in four plants per water treatment per chamber. For the high water treatment (W_H), plants were provided with three watering events of 8 min per day, during which 252.4 mL was applied via an automated irrigation system and drip emitters (Supertif–PLASTRO, Kibbutz Gvat D. N. Ha'Amakim, Israel). For the low water treatment (W_L), we used the procedure described in Nemali & van Iersel (2006): when the average volumetric soil water content dropped below a threshold value of 30%, the datalogger initiated a signal that activated a solenoid and all the W_L seedlings in that chamber received 5.25 mL water over 10 s. After a 15 min delay to allow equilibration in the pots, the process repeated until the treatment level was obtained. Photosynthetically active radiation (PAR) was measured outdoors and inside one chamber with quantum radiation sensors (model LI-190SZ; Li-Cor Biosciences, Lincoln, NE, USA). The PAR sensors were mounted on top of the levelled radiation shields. The PAR sensors were connected to the datalogger and values were recorded at the same frequency as temperature. All equipment and sensors within the chambers were placed in identical locations to further minimize potential chamber effects.

In August 2010, ambient daytime temperatures averaged at 29.5 (±0.8) °C (Fig. 1a). Over the same time period, the elevated heat treatments T_{A3}, T_{W6} and T_{W12} averaged 2.9, 3.2 and 3.1 °C, respectively, above the ambient heat treatment T_{A0} (each significantly different from T_{A0}, $P < 0.05$, but not from the other elevated treatments; Fig. 1b). Mean atmospheric $[CO_2]$ values were 385 μmol mol^{-1} for C_A and 704 μmol mol^{-1} for C_E. The mean soil water content was 28.0% v/v for the low water treatment (W_L) and 50.8% v/v for the high water treatment (W_H) and did not significantly differ among $[CO_2]$ and temperature treatments (Fig. 1c).

Foliar gas exchange parameters

To determine the effect of heat waves on foliar gas exchange, measurements were made prior to, during and after one midsummer heat wave treatment period. Measurements were conducted twice per day (09:00 and 15:00 hours) on four randomly selected seedlings from each of the sixteen treatment combinations. This was done on 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). Measurements were performed
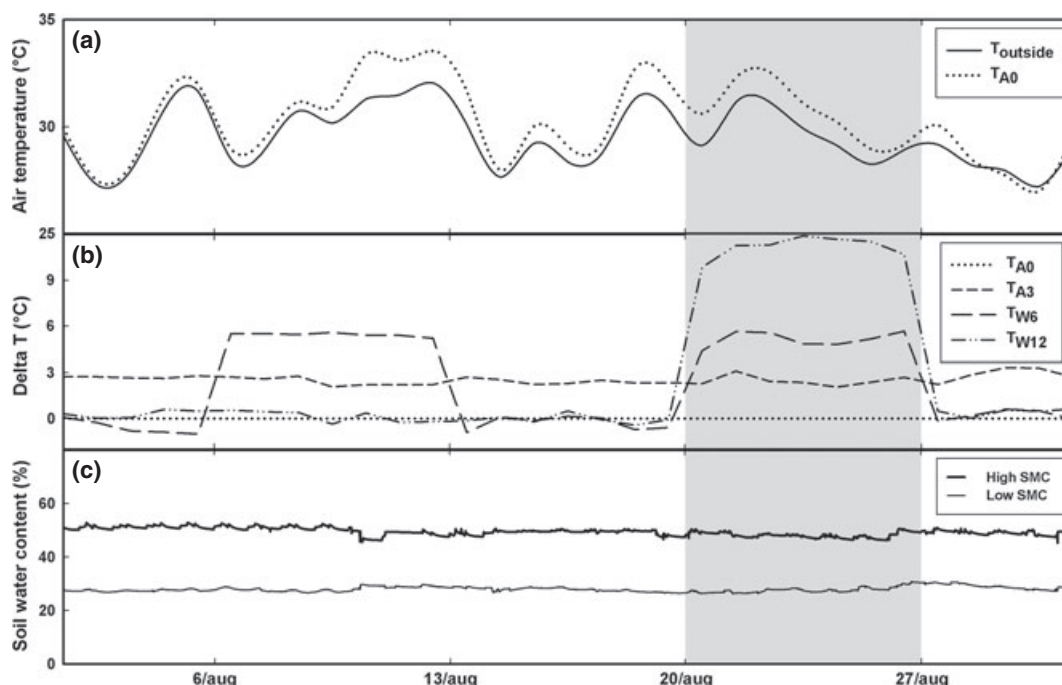
Fig. 1 Temperature and soil water content during the experiment in August 2010. A heat wave was applied from 20 through 26 August (grey area). (a) mean daily ambient air temperature at 1 m above the ground outside ($T_{outside}$) and inside the ambient treatment chambers (T_{A0}); (b) mean daily temperature differences (Delta T) between the temperature of the ambient temperature treatment (T_{A0}) and the constant + 3 °C elevated heat treatment (T_{A3}), the moderate heat wave treatment (T_{W6}) or the severe heat wave treatment (T_{W12}); (c) mean hourly soil water content for the high soil water treatment (W_H) and the low soil water (W_L) treatment, averaged across both [CO_2] treatments.

on sunny or mostly sunny days. All measurements were conducted on foliage from the most recent fully developed flush.

Foliar gas exchange parameters were measured using a portable photosynthesis system (model LI-6400; Li-Cor Biosciences, Lincoln, NE, USA) fitted with a fluorescence head (6400-40 Leaf Chamber Fluorometer, Li-Cor Biosciences, Lincoln, NE, USA). Light saturated net photosynthesis (A_{net}, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were simultaneously measured on the selected seedlings. The chamber temperature of the fluorescence head was set to match the actual temperature measured in the treatment chambers at the start of the measurement. This means that measurement temperatures varied across different days as a function of ambient temperature (Fig. 1a), as treatment temperature differences were consistently maintained (Fig. 1b). The light source of the fluorescence head was maintained at 1500 μmol photons m^{-2} s^{-1} and [CO_2] was maintained at treatment conditions, either 380 or 700 μmol CO_2 mol^{-1} for C_A and C_E respectively.

Growth measurements

Seedlings were harvested at the end of the growing season (2 October 2010) just before leaf senescence. At this stage, the seedlings had undergone five heat wave cycles. Roots were hand washed to remove all potting material. Foliage, stem and

root biomass of 15 seedlings per treatment were dried at 60 °C for 1 week and weighed.

Data analysis

There were a total of 16 treatment combinations in this study: two [CO_2] treatments × four heat treatments × two water treatments. Measured and calculated parameters were analysed using a three-way repeated measures analysis of variance (RMANOVA) with treatment day (nine levels; two before, four during and three after the heat wave) and time of day (two levels; 09:00 and 15:00 hours) as fixed repeated factors on a physiological parameter (A_{net}, g_s or E). Biomass values were analysed via three-way ANOVAs to account for [CO_2], heat and soil water treatments. Tree ($n = 4$) was included as a random effect in all analyses. Holm-Sidak's multiple comparison test was used to determine differences among treatments in each parameter. Statistical tests were performed using proc mixed model analyses in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and R (R Development Core Team, 2009).

Results

Cumulative effect of heat treatments on growth

The heat treatments significantly affected leaf, root and total biomass (Table 1). However, there were no

interactions between temperature treatments and the $[CO_2]$ and soil water treatments on total or component biomass. Averaged across all $[CO_2]$ and soil water treatments, total biomass at the end of the experiment was 23% lower in T_{W12} than T_{A3}, even though both treatments had the same average temperature and received the same heat sum (Fig. 2a). Total biomass was not significantly different among the T_{A0}, T_{A3} and T_{W6} treatments.

Total and component biomass was significantly affected by the C_A and C_E treatments (Table 1). Across all heat treatments combined, and for T_{A0} and T_{W12}, total biomass was greater in C_E than C_A in every heat treatment (Fig. 2b). When compared within $[CO_2]$ treatment level, in C_A biomass was 40% lower in T_{W12} than in T_{A3} and 25% lower in T_{W12} than in T_{A0}, but in C_E, there were no significant differences in biomass among the four heat treatments.

Total and component biomass was significantly affected by the W_H and W_L treatments (Table 1). For every heat treatment, total biomass was substantially greater in W_H than in W_L (Fig. 2c). The differences in total biomass between W_H and W_L ranged from 53% in T_{A0} to 63% in T_{W6}. Within the W_H treatment, biomass was significantly lower in T_{W12} than T_{A3}. Within the W_L treatment, biomass was not significantly different among any of the heat treatments.

Comparing among heat treatments within CO_2 and soil moisture levels, we found that within the C_AW_H treatment combination, total biomass was significantly reduced in both heat treatments compared to the T_{A3} control (Fig. 2d). The mean biomass of $C_AW_HT_{A3}$ was 100.8 (±11.0) g. The severe heat wave ($C_AW_HT_{W12}$) reduced this value by 39% ($P < 0.001$). Biomass in T_{W12} was also significantly less than in T_{A0}. However, in C_EW_H, there were no differences among the heat treat-

ments. In both C_A and C_E, the W_L treatment substantially reduced total biomass, and there were no significant differences among heat treatments in W_L with the exception of $C_AW_LT_{W12}$, which had significantly lower biomass than T_{A0} and T_{A3}.

Effect of heat treatments on net photosynthesis

Net photosynthesis was 42% greater in C_E than in C_A, averaged across all measurements (pre-, during- and post-heat wave, morning and afternoon, W_H and W_L combined; Table 1, Fig. 3). Within each $[CO_2]$ treatment, all of the elevated heat treatments had lower values of A_{net} than T_{A0}. The A_{net} of the T_{W6} and T_{A3} treatments were similar, although only the A_{net} of T_{A0} and T_{A3} were significantly higher than T_{W12}, and only in C_A.

Comparing A_{net} of the T_{A0} and T_{A3} treatments on individual days showed that they responded differently in C_A and C_E (Fig. 4). In C_A, both control treatments had higher A_{net} in W_H than W_L, and within each water regime A_{net} of T_{A0} and T_{A3} were similar. However, in C_EW_H, A_{net} of T_{A0} was often substantially higher than in T_{A3}, but in C_EW_L this pattern was reversed and T_{A3} was substantially higher than T_{A0}. It is also interesting that in W_H, A_{net} of T_{A0} was substantially higher in C_E than C_A, but A_{net} of T_{A3} was essentially the same in C_E and C_A.

In C_AW_H, the heat wave treatments had no effect on A_{net} (Fig. 4). However, in C_EW_H, both heat wave treatments had lower A_{net} on Day 7 of the heat wave than before it started, and they showed lower A_{net} than T_{A0}. In C_AW_L, there were no significant differences in A_{net} among the heat treatments, except for Day 1 of the heat wave, when both the T_{W6} and T_{W12} treatments had higher A_{net} than the T_{A0} and T_{A3} controls. In C_EW_L,

Table 1 Linear mixed model analysis of variance (F values) for the $[CO_2]$ (C), heat (T) and soil water (W) treatments, and their interactive effects on gas exchange and biomass growth of 1-year-old northern red oak (*Quercus rubra* L.) seedlings grown under two CO_2 levels, four heat treatments and two soil water contents

Factor	Gas exchange			Biomass (dry mass)			
	A_{net}	g_s	E	Leaf	Stem	Root	Total
C	50.87***	120.88***	102.42***	20.07***	17.28***	31.36***	27.55***
T	3.40*	2.87*	2.86*	3.46*	1.32	6.79***	4.54**
W	24.37***	46.80***	42.98***	157.97***	155.72***	196.57***	214.19***
C × T	0.27	2.46	0.24	2.07	2.29	1.24	1.59
C × W	2.54	17.90***	15.67***	0.96	4.89*	1.64	2.44
T × W	7.04***	3.52*	1.59	0.78	0.72	0.75	0.91
C × T × W	3.41*	1.00	0.84	0.19	1.47	0.38	0.47

Gas exchange abbreviations: A_{net}, net photosynthetic rate; g_s, stomatal conductance; E, transpiration rate. Significance:*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
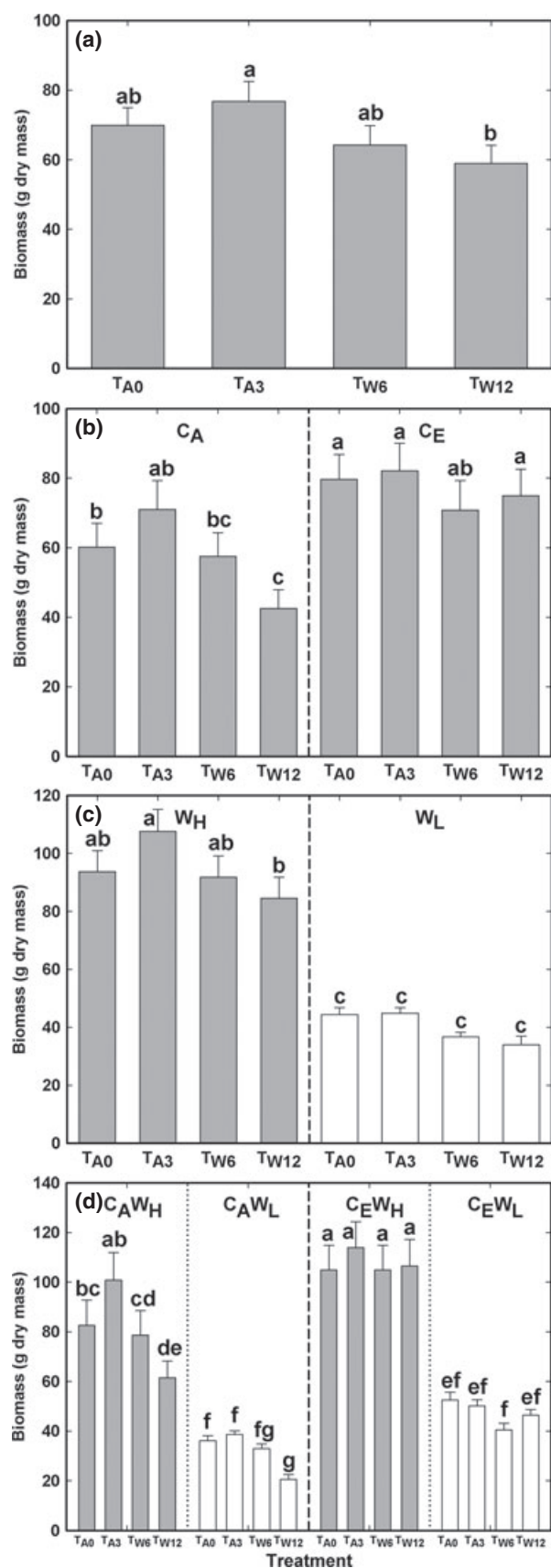
Fig. 2 Mean total biomass (+ SE) of *Quercus rubra* seedlings at the end of the growing season for the four heat treatments: ambient temperature (T_{A0}), ambient + 3 °C (T_{A3}), +6 °C heat wave (T_{W6}) and + 12 °C heat wave (T_{W12}). (a) overall heat treatment means (all other treatments combined); (b) heat treatment means separated by ambient (C_A) or elevated (C_E) CO_2 treatment level; (c) heat treatment means separated by high (W_H) or low (W_L) water treatment level; and (d) heat treatments means separated by CO_2 and water treatment levels. Significant differences ($P < 0.05$) across all bars are denoted by different letters.

there were no differences in A_{net} among T_{A3}, T_{W6} and T_{W12} across the measurement period, but all three of the elevated heat treatments had higher A_{net} than T_{A0}. Relative to pre-heat wave A_{net} values, in W_H there was a significant treatment by time interaction, i.e. as the T_{W12} heat wave progressed, A_{net} declined (Table 1). This was also evident in T_{W6} in C_E, but not C_A. In W_L, this response was less evident.

As is commonly observed in diurnal measurements, the mean A_{net} was always higher in the morning than the afternoon, but when the reduction was <30%, it was generally not statistically significant (Fig. 5). However, there was an interesting response in the T_{W12} treatment. During the heat wave, in the W_L treatment, the morning values of A_{net} were higher than before or after the heat wave, when the air temperature was at ambient, and also higher than the morning W_H values. In that treatment, there was also a very substantial reduction in A_{net} in the afternoon, declining by 65% and 61% compared with morning values in C_A and C_E respectively.
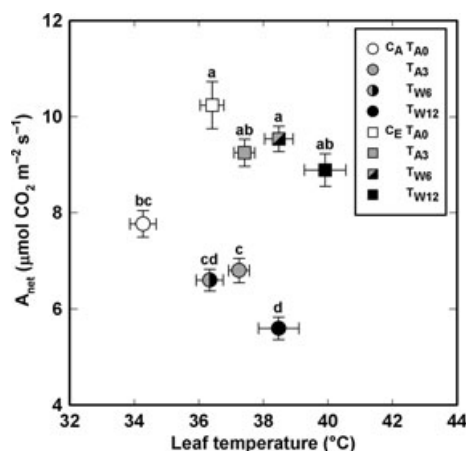

Fig. 3 Mean values and SE of net photosynthesis (A_{net}) and measurement leaf temperature of 1-year-old *Quercus rubra* seedlings grown under two CO_2 levels: ambient (C_A, circles) and elevated (C_E, squares), and four heat treatments: ambient temperature (T_{A0}), ambient + 3 °C (T_{A3}), +6 °C heat wave (T_{W6}) and + 12 °C heat wave (T_{W12}). Values were averaged across the entire experiment.

Effect of heat treatments on stomatal conductance and transpiration

A comparison of mean g_s and E across the measurement period, i.e. pre-, during- and post-heat wave measurements combined, showed that in all heat and water treatments both g_s and E were greatly reduced in elevated $[CO_2]$ compared with ambient $[CO_2]$ (Fig. 6). In C_AW_H, g_s was significantly reduced in T_{A3}, T_{W6} and, especially, T_{W12} compared with T_{A0}, but there were no significant differences in g_s among the heat treatments in C_EW_H. Although g_s was reduced in the heat treatments under C_AW_H, E was unaffected. Under C_AW_L, E was higher in T_{W12} than T_{A0}, and statistically similar among the three elevated heat treatments. In C_E in either W_H or W_L, g_s and E were not different among heat treatments. Within heat treatments in C_E, only in T_{A0} were g_s and E lower in W_L than W_H.

Averaged across CO_2 and water treatments, there was no significant difference in g_s among the four temperature treatments on each measurement day either before, during or after the heat wave (Fig. 7). However, g_s rose 29% on the first day of the heat wave in T_{W6}, and 32% in T_{W12}, compared with before the heat wave. However, in both treatments this difference disappeared on Day 7 of the heat wave. After the heat wave, g_s dropped to its pre-heat wave level.

Transpiration increased significantly during the heat wave period in both T_{W6} and T_{W12} compared with pre-treatment values (Fig. 7). The largest effect was observed in the T_{W12} treatment where E increased by 95% on Day 1 of the heat wave compared with the Before value (from 2.20 to 4.29 mmol H_2O m^{-2} s^{-1}). After the heat wave, E was 28% lower than before the heat wave.
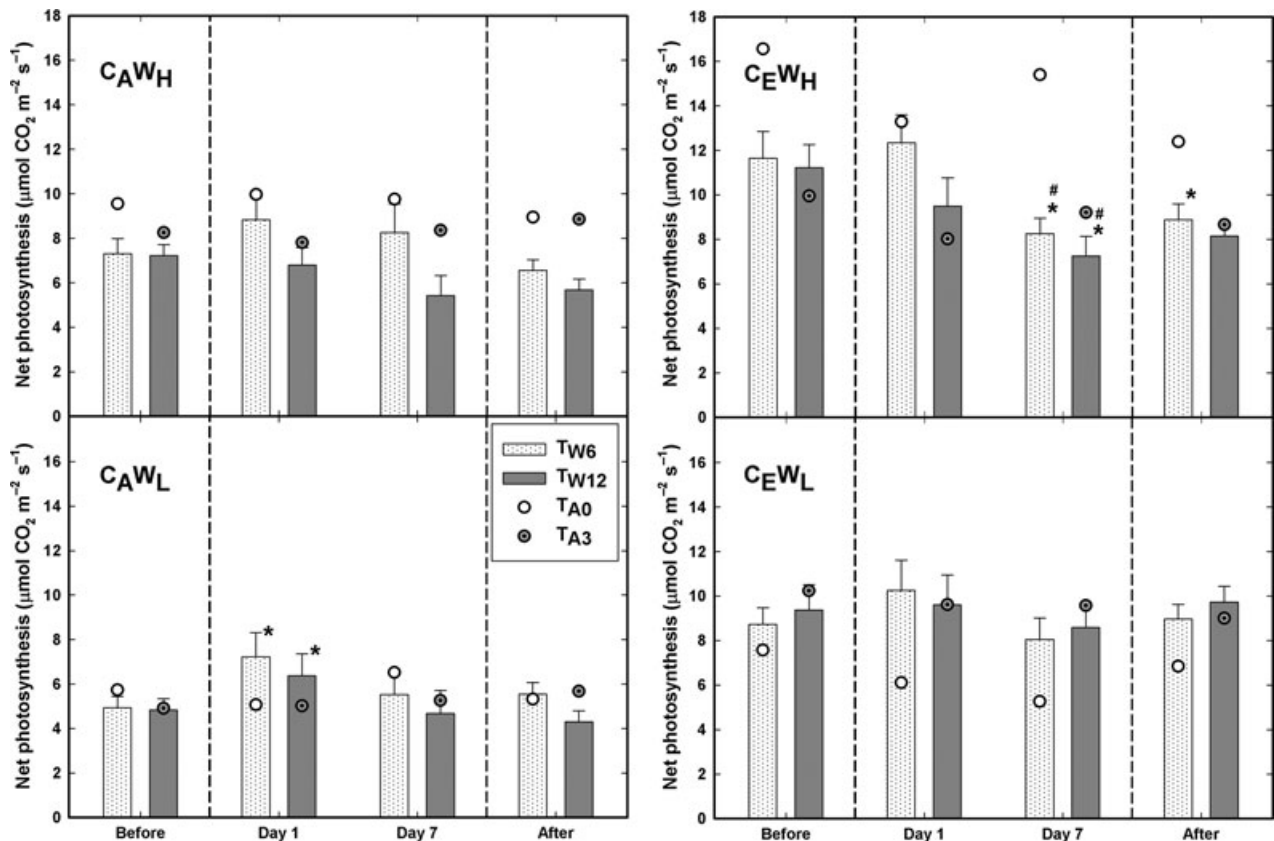

Fig. 4 Mean (+ SE) net photosynthesis of *Quercus rubra* seedlings grown under two CO_2 levels: ambient (C_A, left panels) and elevated (C_E, right panels), four heat treatments: ambient temperature (T_{A0}), ambient + 3 °C (T_{A3}), +6 °C heat wave (T_{W6}) and + 12 °C heat wave (T_{W12}), and two soil water contents: high (W_H, top panels) and low (W_L, bottom panels). Values are both morning and afternoon measurements, averaged before a week long heat wave in August 2010 (Before), after the heat wave (After), and on the first (Day 1) and the last day (Day 7) of the heat wave. Heat wave treatments T_{W6} and T_{W12} are depicted as bars, controls T_{A0} and T_{A3} as circles. Significant differences ($P < 0.05$) with a treatment's average *before* the heat wave are depicted by an asterisk *, whereas a significant difference ($P < 0.05$) between the first and the last day of the heat wave is depicted by a hash tag #.
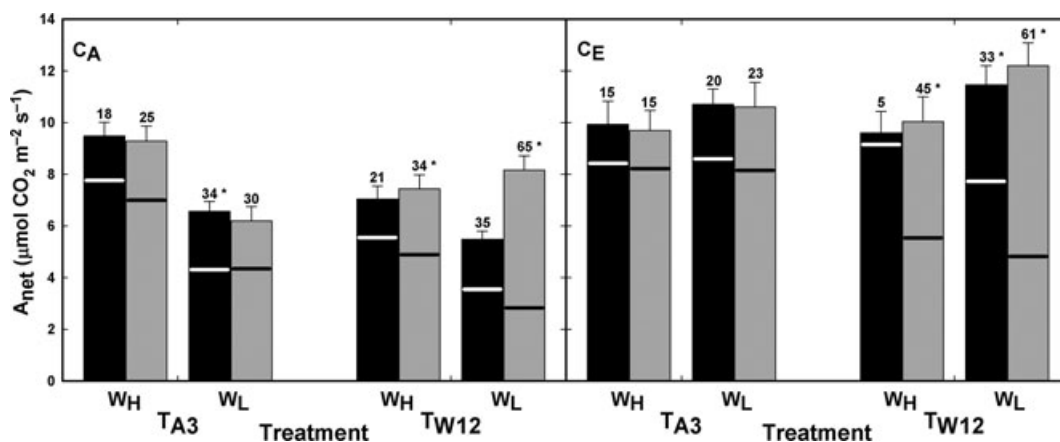
Fig. 5 Mean (+ SE) net photosynthesis (A_{net}) of *Quercus rubra* seedlings grown under two CO_2 levels: ambient (C_A, left panel) and elevated (C_E, right panel), and two heat treatments: control (T_{A3}) and heat wave (T_{W12}) and two soil moisture contents: high (W_H) and low (W_L), *before + after* (black bars) or *during* (grey bars) an imposed heat wave period in August 2010. Morning measurements (09:00 hours) are represented by vertical bars, afternoon measurements (15:00 hours) are represented by horizontal markings within each bar. The percentage decrease from morning to afternoon is shown above each bar. An asterisk * denotes when this decrease was significant ($P < 0.05$). Values are either averaged across all measurements *during* or *before + after* the heat wave.

Discussion

The results from this study largely supported our hypotheses. We found that the most intense heat wave had the greatest negative effect on gas exchange and growth, supporting our first hypothesis, and that decreased soil moisture exacerbated, and elevated CO_2 mitigated, the negative effects of a heat wave, supporting our second and third hypotheses. Biomass growth integrates temporal variation in physiological processes and is a sensitive indicator of the effects of temperature on plants. We found that heat waves affected biomass growth differently than the same amount of heat applied uniformly over the growing season, supporting our fourth hypothesis, but the response was dependent on the [CO_2] and soil moisture conditions. Under high water availability and ambient [CO_2], we observed that a constant increase of +3 °C increased biomass, which was consistent with many reports of the effects of elevated temperature on tree species (Way & Oren, 2010). We also observed that biomass growth was significantly reduced by the T_{W6} and T_{W12} heat waves, the opposite result from the constant ambient +3 °C treatment (T_{A3}), even though each of these treatments had the same mean temperature and heat sum for the growing season.

The reductions in whole-plant biomass in the heat wave treatments T_{W6} and T_{W12}, compared with the constant elevated heat treatment T_{A3}, were not clearly linked to A_{net} differences in those treatments. For example, even though there were significant biomass differences between T_{A3} and T_{W6} as large as 22%, there was no significant A_{net} difference between T_{A3} and T_{W6} in

any [CO_2] × water treatment combination. This indicates that A_{net} was not the only controlling factor responsible for the growth responses in this experiment. It is likely that the heat treatments affected a combination of many factors integrated over time that contributed to biomass growth. The differences in growth among the four heat treatments disappeared in elevated [CO_2] in both high and low soil water availability. Similar to the findings of others (Hättenschwiler *et al.*, 1997; Saxe *et al.*, 1998), in this study, elevated [CO_2] stimulated biomass growth in all treatments. Usami *et al.* (2001) found a positive interaction of both a +3 °C and a +5 °C increment with [CO_2] on growth in *Q. myrsinaefolia*. Wertin *et al.* (2011) applied a constant +6 °C temperature increase to *Q. rubra* seedlings grown for a growing season in elevated [CO_2] and found a significant reduction in biomass, suggesting that the +6 °C and +12 °C heat waves imposed in this study caused less stress than a constant elevation in temperature, which again emphasizes that the response to heat waves and to constant elevations in temperature are different.

Naudts *et al.* (2011) observed that higher ambient temperatures increased grassland biomass under well-watered conditions, but decreased it under droughted conditions. In our study, low soil moisture conditions led to lower biomass production in all combinations of temperature and [CO_2], but did not have significant interactive effects with those factors. In other words, soil moisture had a large effect on growth, but the relative effect of a temperature and CO_2 treatment combination on growth was consistent in both high and low soil water conditions.
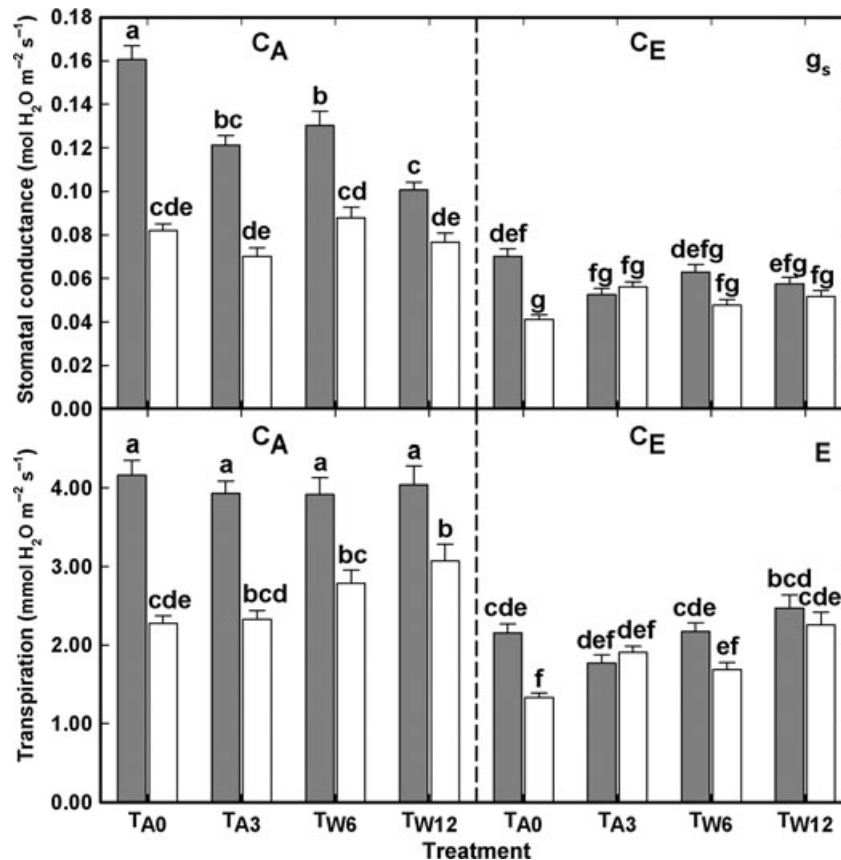
Fig. 6 Mean (+ SE) stomatal conductance (g_s) and transpiration (E) of *Quercus rubra* seedlings grown under two CO_2 levels: ambient (C_A) and elevated (C_E), four heat treatments: ambient temperature (T_{A0}), ambient + 3 °C (T_{A3}), +6 °C heat wave (T_{W6}) and + 12 °C heat wave (T_{W12}) and two soil water contents: high (W_H, grey bars) and low (W_L, unfilled bars). Values were averaged across the entire experiment. Significant differences ($P < 0.05$) across all bars are denoted by different letters.

On the first day of the heat wave, we observed a significant decline in afternoon A_{net} in the T_{W12} treatment which remained evident throughout the heat wave. Similar results were reported for *Larrea tridentata* when exposed to 9 days of extreme heat (Hamerlynck *et al.*, 2000). We did not observe an initial decline in the T_{W6} treatment, which may indicate that the plants may have been able to acclimate to the regular cycles of a +6 °C increase in temperature, or that this elevation in temperature did not exceed the photosynthetic temperature optimum. The behaviour of the T_{W6} and T_{W12} treatments in this study was the same as that of analogous treatments in a parallel study on *Pinus taeda* (Ameye *et al.*, 2012). Thermal acclimation of photosynthesis is not commonly observed in tree species (Ow *et al.*, 2008), but has been reported in *Q. rubra* and four other deciduous tree species (Gunderson *et al.*, 2009), as well as *Larix decidua* (Tranquillini *et al.*, 1986), *Picea mariana* (Way & Sage, 2008) and *Meconopsis horridula* (Zhang, 2010). However, photosynthetic temperature acclimation was not observed in a different study of *Q. rubra*

grown in constant elevated temperature conditions (Wertin *et al.*, 2011).

Elevated $[CO_2]$ substantially increased A_{net} before, during and after the heat wave compared with plants in ambient $[CO_2]$. This compensated for heat wave induced reductions in A_{net}. Elevated $[CO_2]$ reduces photorespiration and thus limitations of the electron transport chain (von Caemmerer, 2000), which restrains A_{net} at very high temperatures (Sage & Kubien, 2007). This has also been observed in *Q. mongolica* (Wang *et al.*, 2008) and several crop species (Taub *et al.*, 2000).

There was a strong effect of water availability on gas exchange, especially on g_s, as well as water, temperature and CO_2 interactions on A_{net} and g_s. Trees grown under lower soil moisture and exposed to the +12 °C heat wave had severely reduced A_{net} in the afternoon, but in the morning they were able to maintain equal or even greater mean A_{net} values to those measured before the heat wave, and comparable morning A_{net} values to well-watered plants. This was particularly apparent in the elevated $[CO_2]$ treatment. The high values of A_{net}
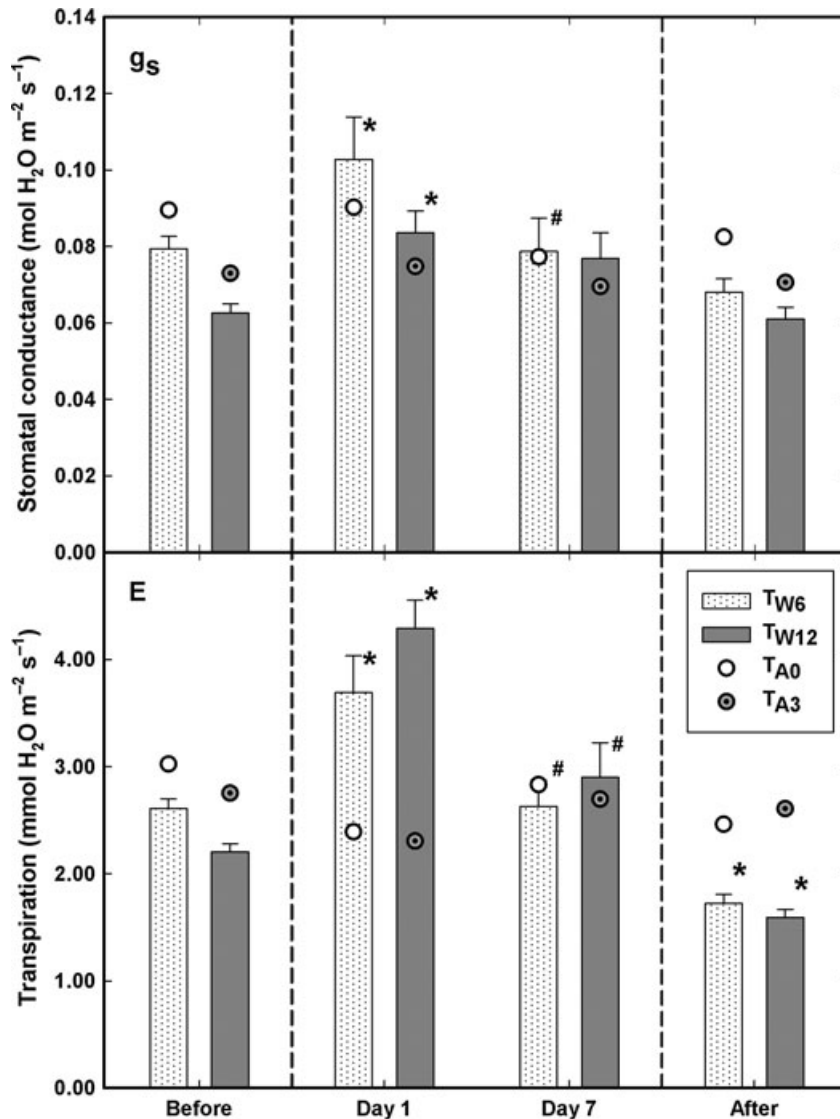
Fig. 7 Mean (+ SE) stomatal conductance (g_s) and transpiration (E) of *Quercus rubra* seedlings during a + 6 °C heat wave (T_{W6}) or a + 12 °C heat wave (T_{W12}). Values are averages before a week long heat wave in August 2010 (Before), after the heat wave (After), and on the first (Day 1) and the last day (Day 7) of the heat wave. Significant differences ($P < 0.05$) with a treatment's average before the heat wave are depicted by an asterisk *. A significant difference between the first and the last day of the heat wave is depicted by a hash tag #. Control treatments of ambient temperature (T_{A0}) and ambient + 3 °C (T_{A3}) are noted by circles.

and g_s in the low water treatment during the heat wave contrasts with the results from desert species subjected to a heat wave (Hamerlynck *et al.*, 2000). However, there have been other reports of increasing values of g_s with rising temperature (Albert *et al.*, 2011; Jin *et al.*, 2011), and empirical indications exist that this increase might be greater under elevated [CO_2] (Allen *et al.*, 2003). The high morning g_s values in plants exposed to the heat wave were particularly evident in the low soil water treatment and may have been an attempt to evaporatively cool the leaves through transpiration (Heath, 1998; Salvucci & Crafts-Brandner, 2004). This was further supported by our transpiration data, which

followed the same pattern as g_s during the heat wave, and we observed that the plants were able to achieve positive photosynthetic rates at leaf temperatures up to 50 °C. Ghouil *et al.* (2003) also reported a drought-induced rise in *Q. suber* thermostability, although only in the short term.

In conclusion, we demonstrated that extreme heat events can affect growth and leaf gas exchange differently than a constant increase in temperature even if both regimes have the same overall average temperature and total heat sum. This suggests that knowledge of the response of plants to a constant elevated temperature may not be sufficient for predicting plant

responses to future conditions if extreme temperature events such as heat waves continue to increase in frequency and intensity. We also observed that elevated [CO$_2$] has the potential to mitigate the effects of heat waves and elevated temperature effects on growth and gas exchange in general. Water availability will remain as crucial factor in determining plant growth and physiological activity in future climate conditions as it is now.

Acknowledgements

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