

# High temperature causes negative whole-plant carbon balance under mild drought

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## Summary

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- Theoretically, progressive drought can force trees into negative carbon (C) balance by reducing stomatal conductance to prevent water loss, which also decreases C assimilation. At higher temperatures, negative C balance should be initiated at higher soil moisture because of increased respiratory demand and earlier stomatal closure. Few data are available on how these theoretical relationships integrate over the whole plant.
- We exposed *Thuja occidentalis* to progressive drought under three temperature conditions (15, 25, and 35°C), and measured C and water fluxes using a whole-tree chamber design.
- High transpiration rates at higher temperatures led to a rapid decline in soil moisture. During the progressive drought, soil moisture-driven changes in photosynthesis had a greater impact on the whole-plant C balance than respiration. The soil moisture content at which whole-plant C balance became negative increased with temperature, mainly as a result of higher respiration rates and an earlier onset of stomatal closure under a warmer condition.
- Our results suggest that the effect of drought on whole-plant C balance is highly temperature-dependent. High temperature causes a negative C balance even under mild drought and may increase the risk of C starvation.

## Introduction

Forests represent large global stores of carbon (C) and account for the largest proportion of annual C exchange with the atmosphere (Phillips *et al.*, 1998; Malhi *et al.*, 1999; Le Quere *et al.*, 2008; Pan *et al.*, 2011; Ballantyne *et al.*, 2012). The C balance of forest ecosystems is sensitive to climate change, which is influenced, for example, by increased growing season duration (Walther *et al.*, 2002), changes in tree growth rates (Clark *et al.*, 2003; Cole *et al.*, 2009) and increased plant respiration and photosynthesis (Piao *et al.*, 2008). There are different means of evaluating the C budget of an ecosystem, such as the widely used eddy covariance technique (Baldocchi, 2003). However, forest ecosystems are made up of individual trees that can respond differently to environmental conditions (e.g. isohydric and anisohydric species; see Tardieu & Simonneau, 1998). Hence, explaining changes in net forest C gain or loss depends on improved understanding of C fluxes at the level of individual trees.

The C balance of an individual plant is the difference between photosynthesis (C input) and respiration (C output). The sum of daytime CO<sub>2</sub> uptake from photosynthesis minus the sum of 24 h CO<sub>2</sub> release through respiration, referred to as the whole-plant daily C balance, is a meaningful indicator of C accumulation within plants (McCree, 1986). Under favorable conditions, a plant takes up more C than it consumes on a daily basis (C surplus) and the excess C is used for growth and synthesis of defense compounds or is stored in the form of nonstructural

carbohydrates for future use, resulting in biomass increase (McCree, 1986; Estiarte & Peñuelas, 1999).

Environmental stresses can alter both photosynthetic and respiratory rates and therefore affect the plant C budget in the short term. The point of zero net C assimilation for the whole plant (i.e. when photosynthetic C uptake equals respiratory C loss on a 24 h basis) is a useful concept to investigate the whole-plant C balance under environmental stresses, and is defined here as the whole-plant C compensation point (CCP).

Under progressive drought, plant growth can be reduced at first (McDowell, 2011) and, as water stress progresses, photosynthesis will be curtailed when stomatal conductance is reduced to prevent water loss through transpiration (Bates & Hall, 1982; Schulze, 1986; McDowell *et al.*, 2008a). However, maintenance respiration may not decline as fast as photosynthesis and the resulting C deficit forces plants to consume stored carbohydrates for respiration (McDowell, 2011). If this C deficit persists, plants will suffer from C starvation as carbohydrates are depleted (Sayer & Haywood, 2006; McDowell, 2011).

At elevated temperatures, enhanced transpiration can hasten the closure of stomata under water stress to prevent water loss (Schulze *et al.*, 1973), simultaneously advancing the decline of photosynthesis. In addition, plants usually present higher maintenance respiration at higher temperatures (Atkin *et al.*, 2000; Atkin & Tjoelker, 2003), which, combined with reductions in photosynthesis, could lead to a rapid depletion of C storage pools (McDowell, 2011). Some authors suggest that, under elevated

temperature and drought, plants could be threatened by, or even die from, C starvation rather than hydraulic failure (McDowell *et al.*, 2008a; Sala *et al.*, 2010; Hartmann, 2011; McDowell, 2011). In support of this hypothesis, Adams *et al.* (2009) provided evidence that under drought stress, leaf-level respiratory C consumption by pinyon pines was significantly higher at elevated temperature (+4°C) than under ambient temperature. However, difficulties of scaling leaf-level observations of gas exchange to the whole plant did not allow quantification of the net C balance of these trees, owing to the mismatch between leaf-level CO<sub>2</sub> exchange measurements and actual biomass responses (Evans & Dunstone, 1970; Wardlaw, 1990). Responses of plants to drought and high temperature have been mostly studied at the tissue level, while changes in whole-plant C balance have rarely been studied (but for studies in some crops, see McCree, 1986; Miller *et al.*, 2001). To our knowledge, the combined effects of elevated temperature and drought on the whole-plant CCP have not been assessed.

In this study, we carried out a water stress experiment on *Thuja occidentalis* L. at three different temperatures (15, 25 and 35°C), and focused on tracking variations of the components (i.e. photosynthesis and respiration) of whole-plant daily C balance as soil moisture declined. *T. occidentalis* is a drought-tolerant evergreen coniferous species in the Cupressaceae family (Collier & Boyer, 1989). It grows in a wide range of moisture conditions, from swamps to cliff edges, making it an interesting choice for drought studies (Beals, 1965; Collier & Boyer, 1989; Kelly & Larson, 2003; Harlow *et al.*, 2005). The average temperature of its natural habitat usually ranges from -12 to -4°C in winter and from 16 to 25°C in summer (Fowells, 1965). Our hypothesis was that trees would be forced into a negative C balance at higher soil moisture content when grown under high temperature (>30°C) because of higher respiratory demand combined with reduced photosynthesis driven by earlier stomatal closure. At lower temperatures, trees should survive longer and reach the CCP at lower soil moisture contents. The results improve our understanding of the effects of environmental drivers on the whole-plant C balance, with implications for the mechanisms underlying drought-induced tree mortality.

## Materials and Methods

### Materials

Four-year-old trees of *T. occidentalis* L. (half-siblings) purchased from a regional nursery were transferred to plastic pots (14.5 cm diameter × 16 cm high) and kept well watered outdoors for *c.* 10 months. The trees were planted in a C-free mixture of vermiculate and sand (volumetric ratio 2:1) without any added organic matter. This ensured that CO<sub>2</sub> released from the soil was solely derived from roots in our short-term experiment. Nutrients were supplied with a single application of instant fertilizer (Manna Wuxal Super 8-8-6 with microelements; Wilhelm Haug GmbH & Co. KG, Düsseldorf, Germany), combined with a slow-release conifer fertilizer (Substral Osmocote 11-8-17; Scotts Celflor GmbH, Mainz, Germany). In July, 2012, nine healthy

individuals (height from stem base = 58 ± 3 cm, diameter at stem base = 1.0 ± 0.2 cm) were randomly chosen and transferred into airtight cylindrical transparent chambers (17 cm diameter × 80 cm high, made of methyl methacrylate resin) connected to a measurement system to determine CO<sub>2</sub> and H<sub>2</sub>O exchange. The chambers were placed in a climate chamber to control temperature and light. A plastic lid (acrylonitrile butadiene styrene resin) covered each pot and was used to separate the chamber into above- and below-ground compartments. The tree stem passed through center of the cover and was fitted with an airtight seal.

### Experimental design

Three trees were randomly assigned to each of the three different temperatures (15, 25 and 35°C). A cycle of 12 h daylight, which was supplied by halogen lamps with a constant photosynthetically active radiation of 390 ± 10 μmol m<sup>-2</sup> s<sup>-1</sup> (the approximate light saturation point of *T. occidentalis*, Matthessears & Larson, 1991) measured inside the chambers at the top, was followed by 12 h of darkness. Air and soil temperatures within the chambers were kept constant during the experiment in each temperature treatment. As lighting had a heating effect on the temperature inside the chambers, the temperature outside the chambers was down-regulated 3–4°C during the daytime to maintain constant temperature inside the chambers. The growth substrate was watered to achieve field capacity (volumetric water content *c.* 50%) at the beginning (data from the first day at 25°C were missing as a result of technical failure), and pots were then left to dry with no further moisture addition until the end of the experiment. Trees were kept well watered and under their corresponding temperature treatment for 2 d before the experiment started to let them acclimate to the experimental conditions. Because of limited room in the growth chamber, the three temperature treatments were conducted in three separate trials and lasted for 34, 30 and 11 d, respectively. Trees from the different trials were not significantly different in size (height and basal stem diameter) or phenology at the start, to ensure comparability among trials. The experiment ended when transpiration approached zero (i.e. below 0.03 mol H<sub>2</sub>O d<sup>-1</sup>). At the end, the foliage of all the trees was brown and dry.

Ambient air with constant moisture ([CO<sub>2</sub>], 400 ± 20 ppm; δ<sup>13</sup>C-CO<sub>2</sub>, -9.2 ± 0.5‰; and vapor pressure deficit (VPD), 1.02, 1.90 and 3.94 kPa for 15, 25 and 35°C, respectively) was continuously pumped through above- and below-ground compartments of the chambers at constant rates (above ground, 5.5, 6.5 and 9.5 l min<sup>-1</sup>; below ground, 1.0, 1.0 and 1.5 l min<sup>-1</sup> for 15, 25 and 35°C, respectively), which were controlled by needle valves outside the growth chamber. The difference in flow rates between temperatures was to ensure that the difference in the CO<sub>2</sub> and H<sub>2</sub>O concentrations between incoming and outgoing air was within 60 ppm and 20‰, respectively. CO<sub>2</sub> and H<sub>2</sub>O concentrations of the air entering and leaving each compartment were measured separately with a LI-6262 (Li-Cor Inc., Lincoln, NE, USA) once every hour for 6 min and 40 s, switching from one compartment to the next automatically with electromagnetic

valves. Data were collected with a CR1000 data logger (Campbell Scientific Inc., Logan, UT, USA). Hourly CO<sub>2</sub> and H<sub>2</sub>O fluxes (μmol h<sup>-1</sup>) in each compartment were calculated by

$$F_S = \frac{[S]_o - [S]_e}{22.4} \cdot FR \cdot 60, \quad \text{Eqn 1}$$

where [S]<sub>o</sub> and [S]<sub>e</sub> are the CO<sub>2</sub> (or H<sub>2</sub>O) concentrations (ppm) of the air leaving and entering each compartment, respectively; FR is the air flow rate (l min<sup>-1</sup>) through each compartment; and 22.4 is the molar volume at standard temperature and pressure (l mol<sup>-1</sup>). The hourly fluxes were then summed to obtain a daily value. The daytime (12 h) CO<sub>2</sub> flux from the above-ground compartment was defined as net photosynthesis (*P<sub>n</sub>*) and the night-time (12 h) above-ground CO<sub>2</sub> flux was defined as above-ground respiration. Root respiration was the summed 24 h below-ground CO<sub>2</sub> flux. The sum of root and above-ground respiration on a daily basis is referred to as total respiration. The above-ground H<sub>2</sub>O flux was defined as transpiration and was divided into night-time (*E<sub>n</sub>*) and daytime (*E<sub>d</sub>*) components. Water-use efficiency (WUE) was calculated as *P<sub>n</sub>* divided by the daytime transpiration (*E<sub>d</sub>*) on a daily basis. Stomatal conductance (*G<sub>s</sub>*, mol d<sup>-1</sup>) was estimated by (Jarvis & McNaughton, 1986; Whitehead, 1998; McDowell *et al.*, 2008b)

$$G_s = \frac{E_d}{VPD} \quad \text{Eqn 2}$$

During CO<sub>2</sub> assimilation, a reduction in discrimination against <sup>13</sup>C is considered to be a physiological indicator of drought stress (Farquhar *et al.*, 1989). To reveal the variation of <sup>13</sup>C discrimination as soil water content declines and drought stress increases, discrete air samples were taken from the air flow entering and leaving above-ground compartments every 1–3 d at a fixed time (i.e. 2 h after the light was switched on), and were analyzed for <sup>13</sup>C composition in reference to Pee Dee Belemnite (δ<sup>13</sup>C) with a Delta<sup>+</sup> XL Isotope Ratio Mass Spectrometry (IRMS, ThermoFinnigan, Bremen, Germany). δ<sup>13</sup>C<sub>p</sub> indicates here the <sup>13</sup>C composition of the CO<sub>2</sub> assimilated by trees, computed from the mass balance of CO<sub>2</sub> and <sup>13</sup>CO<sub>2</sub> of the air entering and leaving the chamber:

$$\delta^{13}C_p = \frac{[CO_2]_o \cdot \delta_o - [CO_2]_e \cdot \delta_e}{[CO_2]_o - [CO_2]_e} \quad \text{Eqn 3}$$

where δ<sub>o</sub> and δ<sub>e</sub> represent <sup>13</sup>C composition of the CO<sub>2</sub> in the air leaving and entering the chamber, respectively. We assumed the shift in δ<sup>13</sup>C<sub>p</sub> mainly reflects photosynthetic fractionation.

A dendrometer (self-assembled with an 8 mm potentiometric linear transducer supplied by Megatron Elektronik AG & Co., Putzbrunn, Germany) was installed on the stem of one tree in each trial to measure stem diameter variation in response to drought as a proxy for water capacitance (Zweifel *et al.*, 2005). We report here the difference in stem diameter (μm) at a given soil moisture from initial diameter. Soil moisture and air/soil temperature were

monitored using a ThetaProbe ML2x (Delta-T Devices Ltd, Burwell, Cambridge, UK) and a 100 kΩ NTC Thermistor EC95 (Thermometrics Corporation, USA), respectively.

## Statistics

The stress point (SP) was defined as the first day that transpiration showed a significant decline from the average value of the previous days (*t*-test, *P* < 0.05), and the mean soil moisture value (± SD) at the SP of the three individuals was compared among the three temperature treatments. For the period before the SP, it is assumed that no water stress was imposed and differences in respiration and photosynthesis rates reflected the influence of temperature only. The average values of each variable (i.e. *P<sub>n</sub>*, respiration, transpiration, WUE, net C gain, ratio of respiration to *P<sub>n</sub>* and δ<sup>13</sup>C<sub>p</sub>) before the SP were computed and compared among the three temperature treatments. One-way ANOVA followed by a Holm–Šidák test (*P* < 0.05) was applied to identify instances with statistically significant differences. For the period after the SP, we applied mixed-effects modeling to investigate how soil moisture and temperature influenced the C balance of the plants using net C gain, *P<sub>n</sub>*, or total respiration as dependent variables. Interactions of soil volumetric water content (or *G<sub>s</sub>*) and temperature were included as fixed effects and temperature as random effects to obtain separate intercepts and slopes for each temperature (see Supporting Information, Table S1, for a summary of the models). By modeling the temperature effect on the components of C gain, we aimed to separate the temperature effects on the whole-plant C balance from those of *P<sub>n</sub>* (via stomatal conductance) and respiration.

To determine the CCP under each temperature treatment, a sigmoidal model was fitted to the daily net CO<sub>2</sub> gain as a function of soil moisture, from the starting point to the lowest point:

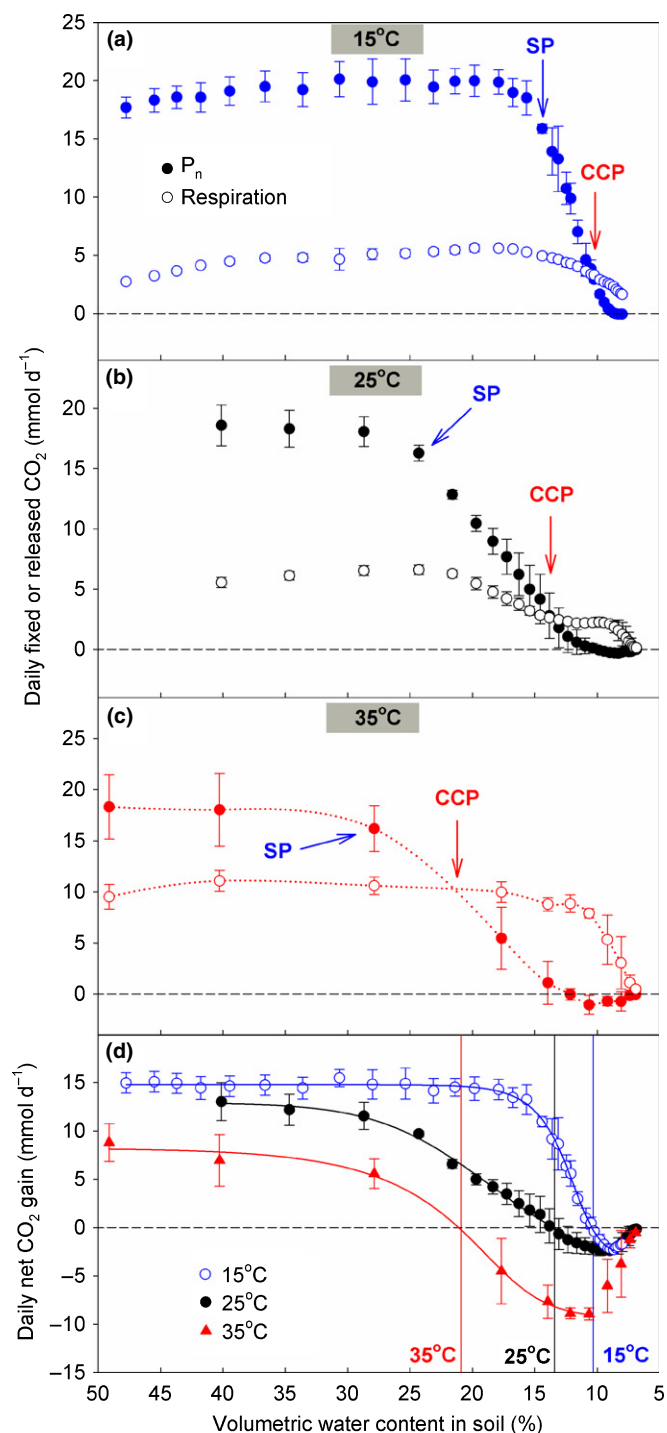
$$y = a + \frac{b}{1 + e^{(c-dx)}} \quad \text{Eqn 4}$$

where *y* is the daily net CO<sub>2</sub> gain, *x* is the soil moisture and *a*–*d* are regression parameters. The point (measured as soil moisture) where net CO<sub>2</sub> gain equals zero was defined as the CCP. All statistics were carried out with R, version 2.15.0 (R Development Core Team, 2012) and the package ‘lme4’ (Bates *et al.*, 2012) was used in the mixed-effects modeling.

## Results

### Progressive drought

*P<sub>n</sub>* and respiration showed similar patterns of response for all three temperature treatments as the soil dried out (Fig. 1a–c). *P<sub>n</sub>* declined sharply after the SP and continued to decrease to values below the respiration rates. Respiration also decreased after the SP but not as fast as *P<sub>n</sub>*. After the CCP, respiration rates showed a rapid decline, eventually reaching values close to zero. Daily net C gain began to decline at the SP and became negative after CCP (Fig. 1d). As total respiration declined to near zero at the end of



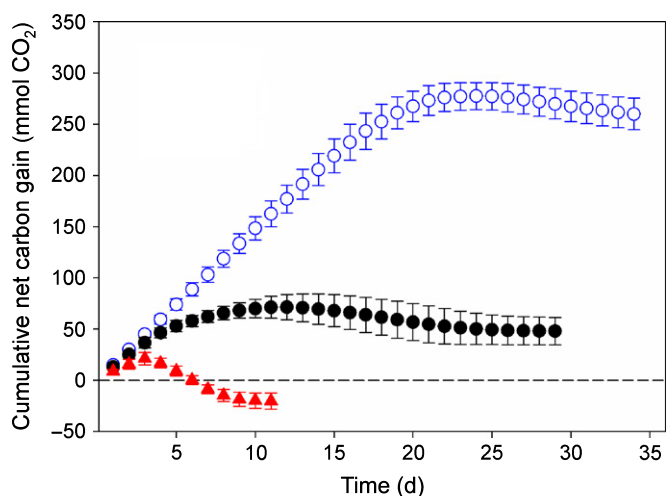
**Fig. 1** Net photosynthesis ( $P_n$ ), respiration (a–c) and net carbon gain (d) of *Thuja occidentalis* as a function of soil moisture under three temperature treatments (15°C, blue; 25°C, black; 35°C, red). In panels (a)–(c), closed circles represent  $P_n$  and open circles represent respiration. Blue and red arrows indicate where we defined the stress point (SP) and the carbon compensation point (CCP), respectively. Smooth (dotted) lines were added in panel (c) to improve visualization because fewer data points were available for the 35°C treatment. In (d) sigmoidal curves (see the Materials and Methods section) were used to fit the data points to estimate the zero point of daily net  $\text{CO}_2$  gain (i.e. CCP, denoted by vertical lines). Bars represent  $\pm 1$  SD (three replicate trees) and bars not visible are within the limits of the symbols.

the experiment, daily C gain increased again and reached approximately zero. As predicted, CCP was shifted by temperature and occurred at higher soil moisture contents (10.3, 13.4 and 20.4%,  $F=31.49$ ,  $P<0.001$ ) as the temperature increased (15, 25, and 35°C, respectively). In particular, 20% soil moisture was still wet enough for trees at 15°C to maintain normal photosynthesis and transpiration, while trees at 35°C with the same soil moisture content were already in net C deficit.  $P_n$  and total respiration at the CCP had already dropped to low rates at 15 and 25°C, while rates at 35°C remained high, suggesting that only mild stress was imposed at this point. Based on total respiration rates at the end of the experiment, trees at 25 and 35°C were dead (zero respiration) after 29 and 11 d, respectively, while trees at 15°C were still alive after 34 d (total respiration =  $1.65 \pm 0.18$  mmol  $\text{CO}_2$   $\text{d}^{-1}$ ) when we ended the experiment.

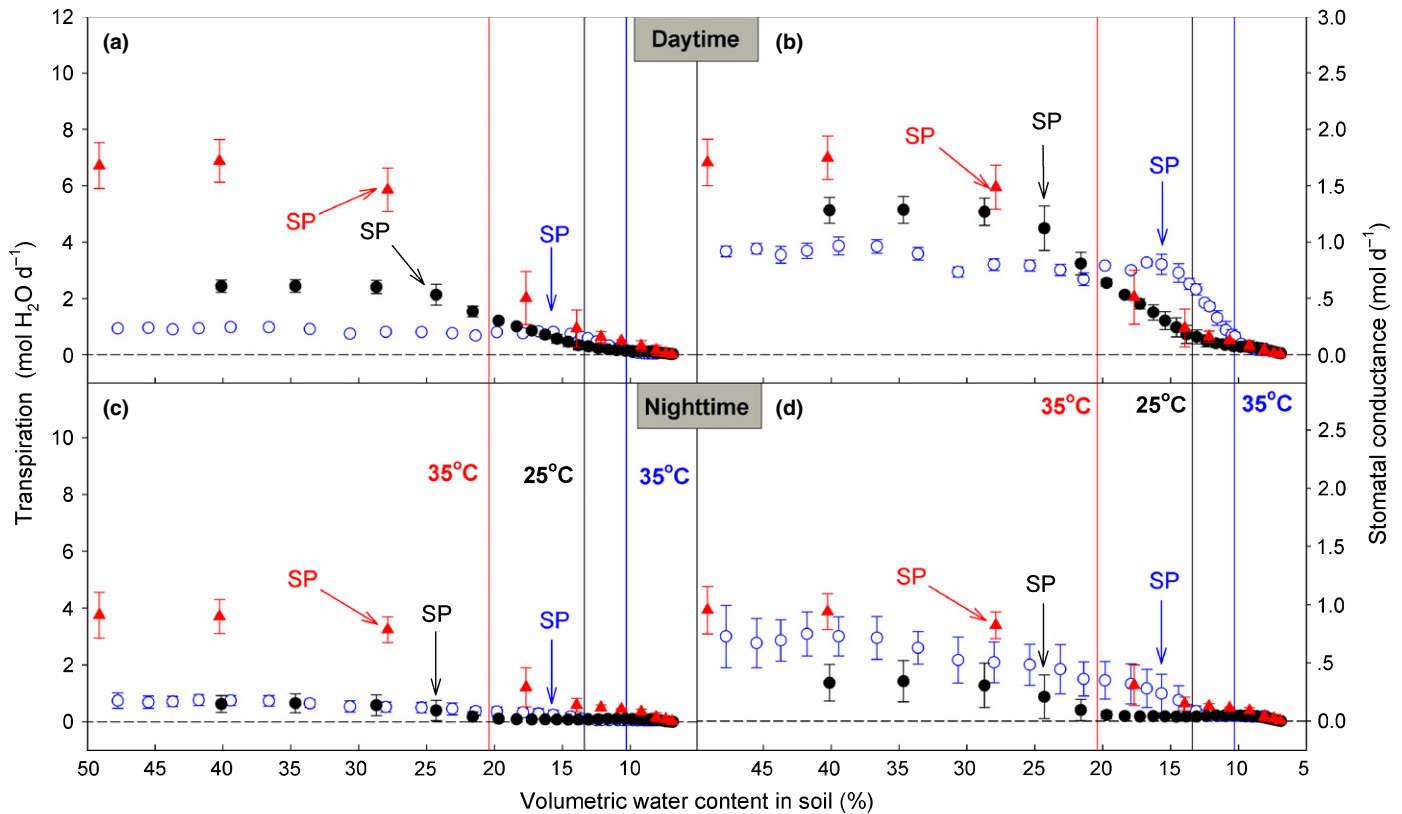
Cumulative net C gain (Fig. 2) showed different patterns among the three temperature treatments. Trees grown at 15°C accumulated much more C than trees grown at 25 or 35°C. At the end of the experiment, trees grown at 15 and 25°C still had not used up the equivalent amount of C accumulated during the experiment. However, trees grown at 35°C had already consumed, within 6 d, as much C as was accumulated during the experiment and seemingly relied on stored C for respiration after that.

After the onset of the SP (Fig. 3a),  $P_n$  declined as a result of decreasing daytime  $G_s$  (Fig. 3b). The higher temperature led to increased transpiration rates and hastened soil drying. Night-time transpiration rates remained low (not exceeding 1 mol  $\text{H}_2\text{O}$   $\text{d}^{-1}$ ) at 15 and 25°C (Fig. 3c). However, at 35°C night-time transpiration was much greater (even higher than daytime transpiration at 15 and 25°C). Night-time  $G_s$  showed a similar pattern for the three temperatures but was slightly lower at 25°C (Fig. 3d).

Water-use efficiency did not drop at the SP, and only dropped after the CCP (Fig. 4a) because  $P_n$  and daytime transpiration



**Fig. 2** Daily cumulative net carbon gains of *Thuja occidentalis* for the three temperatures over the experimental periods, which are 34, 28 and 11 d, respectively, for 15°C (blue circles), 25°C (black circles) and 35°C (red triangles). Bars represent  $\pm 1$  SD (three replicate trees) and bars not visible are within the limits of the symbols.



**Fig. 3** Daytime (above) and night-time (below) transpiration (a, c) and stomatal conductance ( $G_s$ ) (b, d) of *Thuja occidentalis* at different soil moisture content for three temperature treatments. Arrows indicate the stress point (SP) at three temperatures and vertical lines denote the carbon compensation point (CCP) (15°C, blue circles; 25°C, black circles; 35°C, red triangles). Bars represent  $\pm 1$  SD (three replicate trees) and bars not visible are within the limits of the symbols.

decreased proportionally after SP. After CCP,  $P_n$  declined faster than transpiration, leading to the decrease in WUE. Stems of the trees started to shrink after the SP (Fig. 4b), indicating reductions in stem water potential. The increased  $\delta^{13}C_p$  after the SP (Fig. 4c) indicated that stomatal conductance was limiting intercellular  $CO_2$  concentration during C assimilation and induced a decline in isotopic discrimination. Similar to former studies (Farquhar & Richards, 1984; Henderson *et al.*, 1998),  $\delta^{13}C_p$  is well correlated with WUE at the three temperatures (Fig. S1).

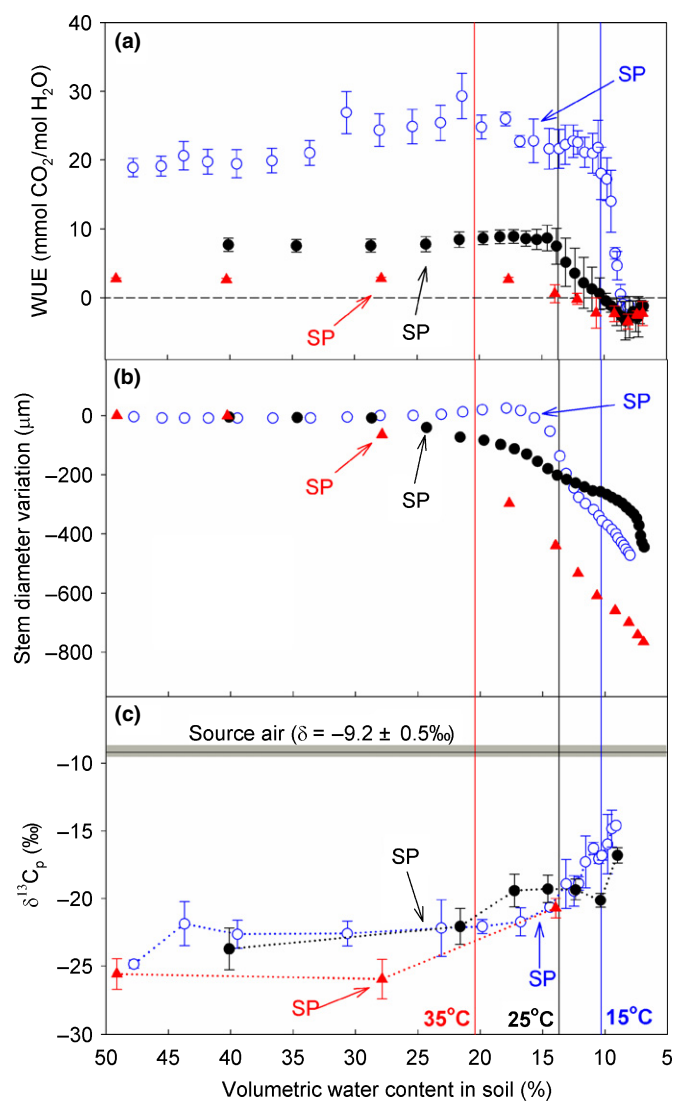
### Effect of temperature without drought stress

The SP, which indicates the onset of stomatal closure, occurred at lower soil moisture at 15°C than at 25 and 35°C (Fig. 5a). Before the SP, that is, before the onset of drought stress, temperature had a significant effect on the C balance. Respiration rates above and below ground increased with temperature and were significantly higher at 35°C (Fig. 5c,d), whereas  $P_n$  was not affected by temperature before the onset of drought stress ( $F=0.224$ ,  $P=0.805$ , Fig. 5b). As a result, daily net C gain was significantly lower at 35°C than at 15°C (Fig. 5e). The ratio of total respiration to photosynthesis was  $\approx 0.24$  at 15°C but reached 0.33 and 0.60 at 25°C and 35°C, respectively (Fig. 5f). Night-time transpiration was much higher at 35°C (Fig. 5g). Daytime transpiration rates increased exponentially with

temperature and were significantly different among temperature treatments (Fig. 5h). WUE dropped significantly as temperature increased and was five to seven times higher at 15°C than at 35°C (Fig. 5i). Similar to transpiration, night-time  $G_s$  was significantly lower at 15 and 25°C than at 35°C, while daytime  $G_s$  increased with temperature (Fig. 5j,k).  $\delta^{13}C_p$  was significantly higher at 15°C than at 35°C (Fig. 5l), indicating that  $CO_2$  availability at the leaf level was not as limited at 35°C as it was at 15°C before the SP.

### Interaction of drought stress and temperature

Temperature showed a significant effect on how the trees' C balance responded to declining soil moisture (Fig. 6). The  $P_n$  of trees grown at 15°C was more sensitive to soil moisture decline after drought stress developed than at higher temperatures (Fig. 6a), whereas response of total respiration to soil moisture was not significantly affected by temperature increase (Fig. 6b).  $P_n$  was more sensitive to soil moisture decline than respiration, making it more of a determinant of the tree C balance during progressive drought.  $P_n$  at lower temperatures was more sensitive to  $G_s$  than at higher temperatures (Fig. 6c), indicating that photosynthesis was more constrained by stomatal conductance at lower temperatures, which in turn made  $G_s$  more important for net C gain at 15°C (Fig. 6d).



**Fig. 4** Water-use efficiency (a), stem diameter (b) and  $\delta^{13}\text{C}_p$  of assimilated  $\text{CO}_2$  (c) of *Thuja occidentalis* as a function of soil moisture content. Arrows indicate the stress point (SP) under three temperature treatments (15°C, blue circles; 25°C, black circles; 35°C, red triangles), and vertical lines denote the carbon compensation point (CCP). (c) The horizontal line and gray area represent the  $^{13}\text{C}$  signature ( $\delta = -9.2 \pm 0.5\text{‰}$ ) of the source air entering the chambers. Bars represent  $\pm 1$  SD (three replicate trees) and bars not visible are within the limits of the symbols.

## Discussion

### Advance of the CCP at elevated temperature

This study revealed that changes in whole-plant C balance during drought stress are highly temperature-dependent. As hypothesized, C deficit occurred in trees at a high temperature (35°C) at a soil moisture content that may not be considered as drought for trees growing at a lower temperature (15°C). Former studies of whole-plant C balance in crops (McCree, 1986; Miller *et al.*, 2001) also suggest that whole-plant C gain is inhibited under water stress and high temperature, but these studies did not separate the temperature and drought effects nor did they determine

whole-plant CCP. Temperature did not affect how plant respiration responded to the progressive drought after the SP (Fig. 6b), while sensitivity of  $P_n$  to soil moisture decline was lower at higher temperatures (25 and 35°C) (Fig. 6a), which itself ran counter to the advance of the CCP at higher temperatures. Therefore, considering initial values before the SP, the earlier occurrence of CCP caused by high temperature was mainly attributed to the higher initial respiration rates (Fig. 5c,d) and an earlier onset of stomatal closure (i.e. SP, Fig. 5a), but not initial  $P_n$  (Fig. 5b).

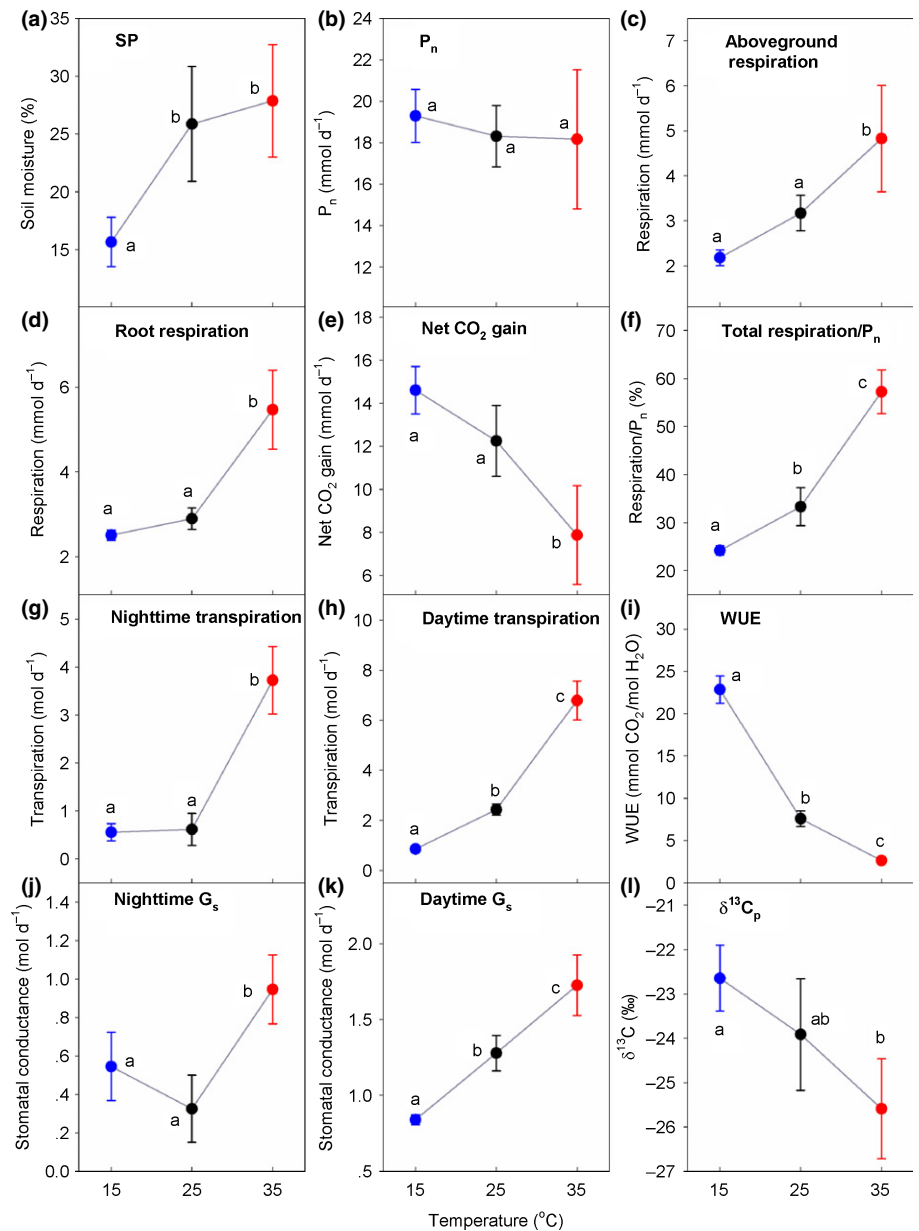
Previous studies in vascular plants have demonstrated that elevated temperatures increase plant maintenance respiration (Atkin *et al.*, 2000; Atkin & Tjoelker, 2003) and hasten stomatal closure as water stress develops (Schulze *et al.*, 1973). These results are consistent with the patterns of the processes that caused the CCP to occur at higher moisture content under elevated temperature. In addition, as temperature rises beyond the optimum point, plant photosynthesis can be reduced (Bernacchi *et al.*, 2001, 2002) and may further advance the CCP during drought/heat events. While the conditions imposed in our treatments may not realistically mirror anticipated patterns of climatic change, our results indicate that the combined effects of drought and elevated temperature have important implications for the C budget of vascular plants under a warmer and drier scenario.

### Temperature effects on plant C balance without drought

Owing to technical constraints, we did not have well-watered trees as a control at each temperature in our experiment, which would have allowed us to separate the effects of temperature from those of declining soil moisture. Instead, we took the period before the SP at each trial as a baseline, assuming no major changes in plant physiology would occur during the experiment under strictly controlled conditions (i.e. without day-to-day variations in temperature and light conditions) if no water stress was imposed.

As temperatures increased from 15 to 35°C, net photosynthetic rates remained constant (Fig. 5b), while respiration increased with a  $Q_{10}$  (the rate of respiration change as temperature increases by 10°C) of *c.* 1.4. Stronger photosynthetic discrimination against  $^{13}\text{C}$  occurred at 35 than at 15°C (Fig. 5l), similar to what has been observed in studies on shrubs under experimental warming (Welker *et al.*, 1993, 2004; Michelsen *et al.*, 1996). This can be explained by the high stomatal conductance at 35°C (Fig. 5k), which kept intercellular  $\text{CO}_2$  concentration high and thereby enhanced C isotope discrimination (Farquhar *et al.*, 1982, 1989). In addition, during the imposed progressive drought, the slopes of  $P_n$  to  $G_s$  (Fig. 6c), reflecting the gradient between ambient and intercellular  $\text{CO}_2$  concentration ( $c_a - c_i$ ) (Farquhar & Sharkey, 1982), were lower at elevated temperatures. This suggests that intercellular  $\text{CO}_2$  concentration was highest at 35°C for a given  $G_s$  value, potentially another reason for the large  $^{13}\text{C}$  discrimination at 35°C.

High VPD is usually considered to have a negative effect on stomatal conductance (Gucci *et al.*, 1996; Wullschlegel *et al.*, 2002), which in turn influences photosynthesis and C balance. Yet, elevated temperature can also widen the aperture of stomata at constant VPD (Schulze *et al.*, 1973). Our results show that



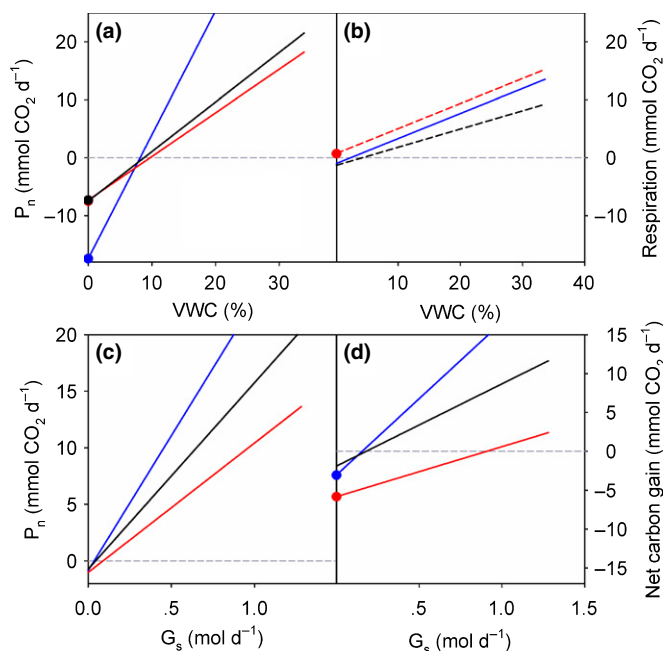
**Fig. 5** The stress point (SP) and values of the other studied variables of *Thuja occidentalis* under nondrought conditions (i.e. average values before the SP) at the three temperatures (15°C, blue circles; 25°C, black circles; 35°C, red circles). Different lowercase letters denote significant differences between the three temperatures (Holm–Šidák test,  $P < 0.05$ ). Bars represent  $\pm 1$  SD (three replicate trees) and bars not visible are within the limits of the symbols.  $P_n$ , net photosynthesis;  $G_s$ , stomatal conductance; WUE, water-use efficiency.

daytime  $G_s$  was higher under warmer conditions (Fig. 5k), indicating that direct temperature effects were much stronger than temperature effects via VPD. However, their individual contributions to changes in stomatal conductance as well as to the C balance need to be quantified in a specifically designed experiment.

#### Effects of temperature and drought on the plant C balance

During the progressive drought,  $P_n$  played a more important role than respiration in the C balance, because  $P_n$  was more sensitive than respiration to soil moisture decline at all temperatures (Fig. 6a,b). Temperature, including its effect on VPD, affected  $P_n$  through  $G_s$  (Fig. 6c), and, hence, the C balance (Fig. 6d). Respiration was primarily directly affected by temperature (Fig. 5c, d), rather than by temperature/drought interactions as indicated by parallel slopes of respiration over soil moisture (Fig. 6b).

We observed two distinct phases of drought stress response. In the first phase,  $P_n$ , respiration, transpiration, stem diameter and  $\delta^{13}C_p$  all started to decline after the SP (Figs 1, 3, 4). However, WUE started to decrease only around the CCP, characterizing the second phase of the response (Fig. 4). Before reaching the CCP, stomatal closure reduced both water loss and CO<sub>2</sub> diffusion into leaves, thereby causing simultaneous reductions in  $P_n$  and transpiration. After the onset of the CCP,  $P_n$  decreased at a greater rate than transpiration, inducing a decline in WUE. One possible explanation is the nonlinear decrease of mesophyll conductance under drought conditions (Flexas *et al.*, 2002), which may impede CO<sub>2</sub> diffusion and induce a sudden decline in C fixation at the CCP. Another possibility is that, regardless of stomatal closure, there could be some metabolic impairment inhibiting CO<sub>2</sub> fixation when plants reach a negative C balance. The rapid decline of respiration after the CCP may also have



**Fig. 6** Coefficients (intercepts and slopes) of mixed-effects models for net photosynthesis ( $P_n$ ) (a, c), total respiration (b) and net carbon gain (d) of *Thuja occidentalis* in response to soil volumetric water content (VWC) or stomatal conductances ( $G_s$ ) at the three temperatures (blue, 15°C; black, 25°C; red, 35°C). Closed circles on the y-axis indicate the intercepts (at 25 or 35°C) that are significantly different from zero (for 15°C) or from 15°C (for 25 and 35°C). Solid lines indicate slopes that are significantly different from zero (for 15°C) or from 15°C (for 25 and 35°C), and dashed lines indicate the insignificant slopes. The significance level is  $P < 0.05$ .

been induced by this (Fig. 1). Flexas & Medrano (2002) demonstrated that stomatal closure is the dominant limitation of photosynthesis under mild and moderate drought conditions, whereas, under severe drought, metabolic impairment (i.e. decreased ribulose-1,5-bisphosphate content) becomes the dominant limitation. Whether the CCP can actually indicate metabolic impairment needs to be further investigated. Given the usefulness of the CCP as an indicator of critical plant C and water relations, further exploration of the physiological processes and potential indicators of the CCP and how these vary with tree species may provide important insights.

### Implications for mechanisms of drought-induced tree mortality

Our results suggest that high temperatures can increase the risk of C starvation by forcing trees to be C-deficient under relatively high soil moisture contents. McDowell *et al.* (2008a) suggested C starvation as a possible cause of drought-induced mortality, especially in warmer environments. In support of this point, Adams *et al.* (2009) demonstrated that higher temperatures increased cumulative respiration of plants at a leaf level under water stress. In our study, at the end of the 15 and 25°C trials, the amount of C that had been accumulated during the experimental period was still far from being depleted (Fig. 2). It seems that these trees were not C-limited at the end of the experiment, even though

respiration rates had dropped to nearly zero. Given the rapid decline in their stem diameter at the end of the experiment (Fig. 4b), indicating a decrease of stem water content, hydraulic failure was the most likely main threat to tree survival at these temperatures. At 35°C, the amount of C respired by the trees exceeded what they had accumulated during the experimental period. At the time of their death, they had consumed up to 27 mmol (324 mg) more C than was accumulated during the experiment. However, given the stem diameter decline (Fig. 4b), hydraulic failure or an interaction between water stress and C deficiency (McDowell, 2011) was probably the cause of the death in these trees. Without sufficient data, we cannot determine the exact mortality mechanism. Still, it is obvious that plants experiencing higher temperatures accumulate less C and are more likely to become C-limited under the same soil moisture conditions.

### Conclusion

With our whole-plant chamber design, we demonstrated that high temperature shifts the CCP of plants to higher soil moisture values. It can be inferred that a warming climate may cause physiological drought (i.e. C deficit) even when no meteorological or severe drought occurs, and may consequently threaten the survival of plants in more regions. Similarly, summer drought may impose a greater threat to plants than droughts during colder periods.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Correlations between  $\delta^{13}\text{C}_\text{p}$  and WUE at the three temperatures.

**Table S1** Summary of the mixed-effects models with temperature (15, 25 and 35°C) as the random effect

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