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Article type : Primary Research Articles

## **The temperature optima for tree seedling photosynthesis and growth depend on water inputs**

Running title: Temperature optima for photosynthesis and growth

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/GCB.14975](https://doi.org/10.1111/GCB.14975)

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Key words: Temperature, rainfall, photosynthesis, stem and root respiration, *Eucalyptus tereticornis*, global warming, forests, biomass

Primary research article

Accepted Article

## Abstract

Understanding how tree growth is affected by rising temperature is a key to predicting the fate of forests in future warmer climates. Increasing temperature has direct effects on plant physiology but there are also indirect effects of increased water limitation because evaporative demand increases with temperature in many systems. In this study, we experimentally resolved the direct and indirect effects of temperature on the response of growth and photosynthesis of the widely distributed species *Eucalyptus tereticornis*. We grew *E. tereticornis* in an array of six growth temperatures from 18 to 35.5 °C, spanning the climatic distribution of the species, with two watering treatments: i) water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ), isolating the direct effect of temperature; and ii) water inputs constant for all temperatures, matching demand for coolest grown plants ( $W_{\text{const}}$ ), such that water limitation increased with growth temperature. We found that constant water inputs resulted in a reduction of temperature optima for both photosynthesis and growth by  $\sim 3$  °C compared to increasing water inputs. Water limitation particularly reduced the total amount of leaf area displayed at  $T_{\text{opt}}$  and intermediate growth temperatures. The reduction in photosynthesis could be attributed to lower leaf water potential and consequent stomatal closure. The reduction in growth was a result of decreased photosynthesis, reduced total leaf area display and a reduction in specific leaf area. Water availability had no effect on the response of stem and root respiration to warming, but we observed lower leaf respiration rates under constant water inputs compared to increasing water inputs at higher growth temperatures. Overall this study demonstrates that the indirect effect of increasing water limitation strongly modifies the potential response of tree growth to rising global temperatures.

## Introduction

Projections of the future terrestrial carbon cycle depend strongly on how global forests are assumed to respond to rising temperature (Mercado *et al.*, 2018, Rogers *et al.*, 2017). Empirical research has reported spatially-divergent growth responses to warming, whereby trees in cold, wet sites typically show an increase in growth, in contrast to trees in warm, dry sites where growth typically declines (Babst *et al.*, 2019, Bowman *et al.*, 2014, Buechling *et al.*, 2017, D'Orangeville *et al.*, 2016, D'Orangeville *et al.*, 2018, Lena *et al.*, 2016, Mäkinen *et al.*, 2002, Price *et al.*, 2013). Identifying the underlying physiological mechanisms responsible for these observed trends is a key to predicting the effect of global warming on tree growth (Medlyn *et al.*, 2011, Reich *et al.*, 2018).

The impacts of warming on plant growth primarily depend on whether plants are above or below their thermal optimum for growth (Drake *et al.*, 2015, Drake *et al.*, 2017b, Reich *et al.*, 2015). A number of studies comparing forest stand growth rates across diverse climates have reported relatively low temperature optima for growth. For example, at the global scale, aboveground biomass carbon density peaked at a mean annual temperature of 8-10 °C (Liu *et al.*, 2014). D'Orangeville *et al.* (2018) reported that the growth rates of boreal tree species in Eastern Canada peaked at an annual mean maximum temperature of 8 – 10 °C. Similarly, low temperature optima for growth have been reported for Australian tall wet eucalypt forests, where diameter growth peaks at a mean annual temperature of 11 °C (Bowman *et al.*, 2014, Prior and Bowman, 2014). Above-ground standing biomass of eucalypt forests also shows a linear declining trend with increasing site mean annual temperature above 11.5 °C without a clear peak (Gordon *et al.*, Wood *et al.*, 2015). It is not clear whether the observed declining trends of tree growth in response to increasing temperature and the comparatively low temperature optima for growth at landscape scale are due to the direct effects of temperature on tree growth (Körner, 2003, Prior & Bowman, 2014) or indirect effects of temperature such as reduced soil moisture availability for growth (Bowman *et al.*, 2014).

Temperature directly affects tree growth by determining the rates of many carbon balance processes, including photosynthesis and respiration (Lambers *et al.*, 2008). The light-saturated photosynthetic rate increases with temperature to a peak, followed by a decline (Berry & Björkman, 1980). Decreases in carbon gain by photosynthesis at temperatures above the optimum may be a potential cause for the decrease in tree growth with warming (Way & Sage, 2008).

However, many studies show that warming tends to increase the temperature optimum for photosynthesis (Hikosaka *et al.*, 2006, Kattge & Knorr, 2007, Kumarathunge *et al.*, 2018). Thus, the low temperature optima for growth at the landscape scale is unlikely to be due to the direct effects of temperature on tree photosynthesis (Drake *et al.*, 2015, Smith & Dukes, 2013). Prior and Bowman (2014) suggested that increased biomass maintenance respiration costs with increasing temperature could be a reason for diminishing growth rates at higher temperatures. A similar hypothesis was proposed by Larjavaara and Muller-Landau (2012) who suggested warming increases the total plant maintenance cost. However, it is unlikely that the observed decline in biomass growth is due to an increase in respiration rates, as there is now strong evidence for thermal acclimation of plant respiration (Atkin *et al.*, 2008, Atkin *et al.*, 2005, Atkin *et al.*, 2000, Crous *et al.*, 2017, Crous *et al.*, 2011, Heskell *et al.*, 2016, Tjoelker *et al.*, 2009, Tjoelker *et al.*, 1999, Tjoelker *et al.*, 2001, Vanderwel *et al.*, 2015), including temperature acclimation of above-ground respiration of trees to warming in field conditions (Drake *et al.*, 2019, Drake *et al.*, 2016)

A potential alternative explanation for the low temperature optimum of growth at the landscape scale is the constraint placed on temperature responses by water availability. Temperature and plant available water negatively co-vary at the landscape scale in a predictable manner due to the temperature-induced decrease in the available water through increased evapotranspiration (Williams *et al.*, 2012, van Mantgem *et al.*, 2009). In the future, it has been predicted that evapotranspiration will increase more than precipitation in many regions of the world in future warmer climates (Kao & Ganguly, 2011). For many regions, climate warming is predicted to occur without any increase in rainfall (Kao & Ganguly, 2011). Hence, with increasing temperature, tree growth may become more constrained by available soil moisture (Allen *et al.*, 2010, Barber *et al.*, 2000, Densmore-McCulloch *et al.*, 2016). Thus, to predict future forest function, it is imperative to quantify how water availability constrains the effects of temperature on plant growth and physiology.

Relatively few studies have separated the direct and indirect effects of warming. Field-based direct air and soil warming experiments conducted under seasonal or inter annual variation of soil moisture provide a test of the interactive effect of warming and soil moisture (Bloor *et al.*, 2010, Butler *et al.*, 2012, Melillo *et al.*, 2011, Reich *et al.*, 2018), but do not allow for the direct and indirect effects of warming to be separated (Volder *et al.*, 2010). In contrast, warming

experiments in controlled environments are generally conducted under well-watered conditions where water addition is increased with warming in order to ensure that plants do not become water limited (e.g. Cheesman & Winter, 2013, Drake *et al.*, 2015, Drake *et al.*, 2017b, Ghannoum *et al.*, 2010, Gunderson *et al.*, 2009, Jarvi & Burton, 2018, Natali *et al.*, 2012, Reich *et al.*, 2016, Slot & Winter, 2018, Smith & Dukes, 2017, Tjoelker *et al.*, 1998, Way & Sage, 2008, Xiong *et al.*, 2000). Although this research demonstrates the direct effects of temperature on tree physiology and growth, it does not quantify the indirect effect of warming on tree growth via changes in plant water demand relative to soil water availability. In experiments where temperature and water availability are manipulated separately, the focus tends to be on how drought modifies the response to warming (Adams *et al.*, 2009, Ayub *et al.*, 2011, Blackman *et al.*, 2017, Crous *et al.*, 2012, Duan *et al.*, 2013, Li *et al.*, 2018), involving a complete cessation of watering and acute water stress. Therefore, further studies are necessary to disentangle the effect of warming on growth under the potentially co-limiting conditions of declining plant water availability typically observed across climate gradients or predicted with climate warming.

In this study, we separate the direct and indirect effects of temperature on plant growth. We experimentally resolved the potential effects of water limitation on the temperature response of growth by growing seedlings of a common and widely distributed tree species (*Eucalyptus tereticornis*) across a wide array of growth temperatures with two watering treatments: (1) water inputs that were increased to match plant and evaporative demand with higher growth temperatures, thus allowing us to quantify the direct effect of temperature alone and (2) constant water inputs, matched to plant demand at the lowest growth temperature, such that warmed plants were subjected to both direct and indirect effects of temperature as water limitation increased with increasing growth temperature. We hypothesised that the temperature optima for photosynthesis and growth would be decreased by the indirect effect of increasing water limitation. We measured key physiological traits including irradiance-saturated leaf net photosynthesis and tissue specific dark respiration rates, and quantified the temperature response of key traits of whole plant growth under the two watering treatments. Our primary objective was to disentangle the direct effects of temperature from the indirect effects of reduced water availability on plant growth.

## Materials and methods

### *Plant material*

Seeds of forest red gum (*Eucalyptus tereticornis* sp *tereticornis*) were obtained from the Australian Tree Seed Centre (CSIRO, Canberra ACT, Australia). The seed source was a forest in Queensland, Australia (15.5S 145.14E) where the mean annual temperature and the mean maximum temperature of the warmest month are 26°C and 32°C respectively. The location receives a mean annual rainfall of 1800 mm with a prominent dry period from December to April. This experiment was a contemporaneous extension of the work by Drake *et al.* (2017b), which demonstrated that widely distributed *E. tereticornis* provenances share a common physiological thermal niche without local adaptation to the climate of origin. Therefore, the temperature and water dependencies discussed here are likely to be independent of the specific seed origin selection. Furthermore, the previous study (Drake *et al.* 2017b) was done entirely at high water availability. We build on this previous work by investigating the indirect effects of temperature on growth via changes in water availability.

### *Experimental design*

Seeds were germinated in a shade house at Western Sydney University, Richmond, NSW, Australia (WSU) (33.62 S, 150.74 E). Seedlings were transferred from tube stock to 7 liter PVC pots filled with a moderately fertile sandy loam soil (with field capacity and the permanent wilting point 0.25 and 0.05 m<sup>3</sup> m<sup>-3</sup> respectively). A detailed description of the soil used is given in Drake *et al.* (2017b). Seedlings were fertilized with a liquid commercial fertilizer (Aquasol, Yates Australia; 250 ml per seedling) fortnightly during the experiment period.

We randomly allocated 30 seedlings to each of six adjacent, naturally sun lit glasshouse rooms (8 m long, 3 m wide and 5 m tall) located at WSU in the Austral summer of 2016 (2016-01-08; defined as day 0). We set the daily mean air temperature of each of six glasshouse rooms (referred to as growth temperature hereafter) to simulate the mean daily summer temperatures across the entire native geographic range of *E. tereticornis* with two extreme growth temperatures outside the range. The six mean daily temperatures were 18, 21.5, 25, 28.5, 32 and 35.5 °C which were achieved through 10 temperature set points with an approximately 9 °C diurnal range during the day-night cycle in all treatments. The corresponding maximum daily air temperatures in each glasshouse room were 24, 27.5, 31, 34, 38, and 41.5 °C respectively.

We maintained room relative humidity (RH) between 60 – 80% in all rooms, such that midday vapour pressure deficit increased approximately from 0.5 kPa (18 °C) to 2.5 kPa (35.5 °C) across growth temperature treatments, reflecting the increase in VPD that occurs with mean annual temperature or warming. VPD in night was comparatively lower compared to day time, which ranged from 0.4 kPa (18 °C) to 1.7 kPa (35.5 °C). Seedlings were watered using an automated irrigation system and soil volumetric water content ( $\theta$ ) was measured hourly in each growth temperature using time domain reflectometers (Campbell Scientific, Logan, UT, USA) installed in four pots within each growth temperature. We recorded air temperature, VPD, RH and photosynthetic photon flux density (PPFD) at canopy height (Apogee quantum sensor, USA) in one-minute intervals day and night and monitored conditions frequently to maintain the desired control levels.

#### *Water input treatment*

After one week of growth under well-watered conditions, 15 seedlings within each growth temperature were assigned to one of the two water input treatments; 1. water inputs held constant for all temperatures, matching plant demand at the baseline growth temperature ( $W_{\text{const}}$ ) or 2. water inputs increased with temperature to match plant demand at each temperature ( $W_{\text{incr}}$ ). We considered the coldest room (room 1, 18 °C) as the baseline growth temperature (i.e. the mean summer temperature of the southern range limit of *E. tereticornis* climate distribution envelope) and maintained equivalent soil water content above 70% of the field capacity for both water input treatments by adding the same quantity of water. In the other rooms (2 to 6),  $W_{\text{const}}$  plants were given the same quantity of water that was added to the plants in room 1. We kept  $W_{\text{incr}}$  plants in other growth temperatures at soil water contents above 70% of the field capacity by adding an adequate amount of water daily throughout the experiment; some variation in soil water content was observed in the  $W_{\text{incr}}$  treatment over time as the irrigation was adjusted to keep up with plant water use (Figure S1). Water treatments were applied using an automated irrigation system coupled with monitoring of volumetric water content via time-domain reflectometry. By doing this we simulated two unique scenarios; 1) an array of temperatures with constant water inputs, such that plant available water decreased with temperature and 2) an array of temperatures where water inputs increased to match plant demand, such that plants were well hydrated. Hence, our unique design allowed us to test the direct temperature effect on plant growth and physiology in

well-watered plants and calculate the indirect effect of temperature mediated through water limitation.

### *Plant growth metrics*

We measured height of the main stem and basal diameter of 15 seedlings per growth temperature per water treatment approximately in weekly intervals. We used an allometric model to estimate total plant dry mass using the measured height and diameter. The allometric model was developed using additional seedlings (both  $W_{\text{incr}}$  and  $W_{\text{const}}$ ) present in each glasshouse room which were periodically harvested throughout this study ( $n=156$ , Drake *et al.*, 2017b). The model is of the form

$$\log_{10}(M_t) = -0.018 + 0.85[\log_{10}(d^2h)] - 0.064[(\log_{10}(d^2h))^2] \quad (1)$$

where,  $M_t$  is the total plant mass (g),  $d$  is the basal diameter (mm), and  $h$  is the stem height (cm).

The model predicted the observed mass with high accuracy ( $r^2=0.98$ ). A detailed description of the allometric model development is given in Drake *et al.* (2017b).

We harvested five randomly selected plants in each growth temperature  $\times$  watering treatment combination at the end of the study between 41-48 days. We separated harvested plants into leaves, stems (including branches and stem tips) and roots which were washed free of soil. These samples were oven dried at 60 °C for 48 h and weighed. We counted the number of leaves of each harvested seedling and measured the total leaf area using a portable leaf area meter (Li-3100C, Licor Biosciences, Lincoln, NE, USA). We calculated mean leaf size for each plant as leaf area  $\text{cm}^2$  / leaf count and specific leaf area (SLA) as leaf area  $\text{cm}^2$ /leaf dry mass g. We calculated leaf, stem and root mass fractions as leaf dry mass/total plant dry mass, stem dry mass/total plant dry mass and root dry mass/total plant dry mass, respectively.

### *Gas exchange measurements*

We measured irradiance-saturated leaf net photosynthetic rate ( $A_{\text{sat}}$ ) on the most recent fully expanded leaf of eight replicate plants in each growth temperature  $\times$  watering treatment combination, using six Licor 6400XT portable photosynthesis systems (Licor Biosciences, Lincoln, NE, USA) with standard 2 $\times$ 3 cm leaf chamber and LED light source (Li-6400-02B LED). Measurements were conducted at a photosynthetic photon flux density (PPFD) of 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$ .

<sup>1</sup> and a flow rate of 500  $\mu\text{mol s}^{-1}$ . We maintained the relative humidity inside the leaf chamber between 60 – 80% and the sample cell  $\text{CO}_2$  concentration at  $400 \pm 5$  ppm. For each growth temperature treatment, we measured  $A_{\text{sat}}$  at a leaf temperature similar to the mid-morning growth temperature (20, 24, 28, 32, 36, and 40 °C respectively). We maintained the leaf temperature at target leaf temperature  $\pm 1.5$  °C by manipulating the chamber block temperature. Measurements were completed between 0930 – 1430 hr of the day. Leaves were allowed at least 10 minutes to acclimate to saturating irradiance and reach steady state before the data were logged. We used Eqn 2 (below) to characterize the temperature response of photosynthesis.

To separate effects of stomatal closure on photosynthesis from other effects of temperature, we applied the one-point method (De Kauwe *et al.*, 2016) to estimate the apparent maximum rate of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) activity ( $V_{\text{cmax}}$ ) at a standard temperature of 25 °C from the  $A_{\text{sat}}$  measurements. Note that this value is not intended to indicate an actual rate of Rubisco activity, but rather to yield a parameter that indicates non-stomatal effects of temperature on photosynthesis. Since the measurements were at steady state, we avoid any issues with applying this method to non-steady-state data (Burnett *et al.* 2019). We also recognize that recent work has criticised a core assumption of all leaf gas exchange measurements, in which the intercellular airspaces of leaves are assumed to be fully saturated with water vapour (Vesala *et al.*, 2017, Cernusak *et al.*, 2018). Given the modest leaf water potentials measured in this experiment, we expect intercellular airspaces to be at or very nearly at full saturation. We follow the standard methodology in the field, but recognize that our estimates of stomatal conductance, intercellular  $\text{CO}_2$  concentration, and  $V_{\text{cmax}}$  are dependent on this assumption.

We measured leaf, stem and root respiration rates for five plants in each water treatment at each growth temperature during the final harvest between 41-48 days. We separated harvested plants into leaves, stems (including branches) and roots, which were washed free of soil and excess water removed using paper towels before measurements. We measured leaf respiration rates ( $R_L$ ) using three randomly selected leaves, combined in a single cuvette for measurement, for each of the eight replicate seedlings. We used the entire stem with branches cut in to 5 cm segments to measure stem respiration rates ( $R_S$ ). We used entire root system or subsample, depending on root mass, to measure root respiration rates ( $R_R$ ). All tissue specific respiration rates were measured at a common temperature of  $25 \pm 1.5$  °C and a reference cell  $\text{CO}_2$  concentration of

400±5 ppm using Licor 6400XT portable photosynthesis systems with the Li-6400-22 conifer chamber. We used a flow rate of 400  $\mu\text{mol s}^{-1}$ , but for roots, it was increased to 700  $\mu\text{mol s}^{-1}$  occasionally due to high moisture content in the measurement chamber. Respiration rates were measured as quickly as possible after harvest (within 1 hour). See Drake *et al.* (2017b) for further details on this measurement protocol on other plants in the same study. After measurements, all samples were oven dried at 60 °C for 48 h, then weighed.

### *Plant water potential*

We measured pre-dawn ( $\Psi_{pd}$ ) and mid-day ( $\Psi_{md}$ ) leaf water potentials on five plants in each growth temperature x watering treatment combination at the end of the study on day 48 using a Scholander type pressure chamber with a maximum range of  $-10$  MPa (PMS Instruments, Corvallis, OR, USA).

### *Temperature response of growth and photosynthesis*

We used a simple mathematical model (June *et al.*, 2004) to characterize the temperature response of plant mass, photosynthesis and other variables whenever it showed a peaked response to growth temperature. The model is of the form:

$$k(T) = k(T_{opt}) \exp\left(\frac{T - T_{opt}}{\Omega}\right) \quad (2)$$

where  $k(T)$  is the process rate at temperature  $T$ ,  $k(T_{opt})$  is the process rate at the optimum temperature  $T_{opt}$  and the  $\Omega$  is the temperature at which  $k$  falls to  $0.37$  ( $e^{-1}$ ) of its value at  $T_{opt}$ .

However, for SLA we fitted a simple linear regression model of the form

$k(T) = \alpha + \beta T$  where,  $\alpha$  is the intercept and  $\beta$  the slope, to describe the temperature response as it showed a linear relationship with growth temperature. For tissue specific dark respiration rates, stomatal conductance and transpiration data, we fitted general additive models (GAM) to characterize the temperature response.

### *Data analysis*

We estimated the parameters of Eqn 2 in a non-linear regression framework using *nls* function within the *nlme* package in R version 3.5.1 (R Development Core Team, 2018). However, for SLA, we used simple linear regression (*lm* function in base R) to describe the temperature response as it showed a clear deviation from the peaked response. We used 95% confidence intervals of the parameter estimates (*confint2* function within the *nlstools* R package) to test the null hypothesis of no significant differences in the temperature response between well-watered ( $W_{incr}$ ) and water-limited ( $W_{const}$ ) treatments. We identified a given parameter as significantly different between two treatments if the parameter values had non-overlapping 95% CIs. However, we further tested the 95% CI-based inferences by using non-linear mixed effect models (*nlme* function within *nlme* R package). Here we compared two models fitted with and without a fixed

parameter effect for water treatment, using the likelihood ratio test, followed by post-hoc pairwise comparisons (*glht* function within *multcomp* R package). For tissue specific dark respiration rates and leaf, stem and root mass fraction data, we fitted general additive models (GAM) (Rigby & Stasinopoulos, 2005) to visualize the patterns with growth temperature. We tested for significant differences in the growth temperature response between watering treatments of these variables by comparing the fitted 95% CI between water treatments. We used standardized major axis regression (SMA; *sma* function within *smatr* R package) to test for significant differences between watering treatments in biomass allocation to different components. SMA is a procedure for assessing heterogeneity of regression slopes which characterizes the best fit bivariate line between two variables (Warton et al., 2012, Zhang et al., 2016). The dataset used for this study is publicly available (Drake *et al.*, 2016b) and the analysis code to reproduce all the results, including the figures and tables, is available at (<https://bitbucket.org/Kumarathunge/great-drought>).

## Results

### *Soil and plant water status*

The mean soil volumetric water content ( $\theta$ ) throughout the experiment in plants where water inputs matched plant demand ( $W_{\text{incr}}$ ) was similar across growth temperatures with a mean of  $0.20 \pm 0.002 \text{ m}^3\text{m}^{-3}$  (Figure 1, Figure S1). Thus, while there was some variation in  $\theta$  over time, this treatment successfully kept plants well-watered across a wide range of growth temperatures. The mean  $\theta$  of plants grown with constant water inputs ( $W_{\text{const}}$ ) decreased with increasing growth temperature ( $T_{\text{growth}}$ ), from  $0.17 \pm 0.01 \text{ m}^3\text{m}^{-3}$  at  $18 \text{ }^\circ\text{C}$  to  $0.09 \pm 0.01 \text{ m}^3\text{m}^{-3}$  at  $35.5 \text{ }^\circ\text{C}$  (Figure 1, Figure S1). As expected given the experimental design,  $\theta$  differed between  $W_{\text{const}}$  and  $W_{\text{incr}}$  plants at all  $T_{\text{growth}}$  treatments except for  $18 \text{ }^\circ\text{C}$ , (Figure S1).

Leaf pre-dawn water potential ( $\Psi_{\text{pd}}$ ) gradually decreased with increasing  $T_{\text{growth}}$  in both water treatments, but the rate of decline was larger in  $W_{\text{const}}$  plants (Figure 2a, major axis regression slopes differ,  $P < 0.05$ ; Table 1). The decreasing trend in  $\Psi_{\text{pd}}$  with  $T_{\text{growth}}$ , even in the well-watered conditions, may reflect night-time transpiration (Ogle *et al.*, 2012). Similar to  $\theta$ ,  $\Psi_{\text{pd}}$  at the coldest  $T_{\text{growth}}$  was not significantly different between  $W_{\text{incr}}$  and  $W_{\text{const}}$  treatments (95% CI overlapped). However,  $\Psi_{\text{pd}}$  was significantly lower in  $W_{\text{const}}$  plants compared to the  $W_{\text{incr}}$  at other growth temperature treatments (Figure 2b). Mid-day leaf water potential also decreased with increasing  $T_{\text{growth}}$  ( $\Psi_{\text{md}}$ ; Figure 2b), Similar to  $\Psi_{\text{pd}}$ , the rate of decline was larger in  $W_{\text{const}}$  compared to  $W_{\text{incr}}$  (Table 1).  $\Psi_{\text{md}}$  of  $W_{\text{const}}$  plants was lower than the  $\Psi_{\text{md}}$  of  $W_{\text{incr}}$  plants at all  $T_{\text{growth}}$  except  $18^\circ\text{C}$ . These results indicate that water limitation increased with temperature in the  $W_{\text{const}}$  treatment group.

### *Plant growth*

We observed strikingly different temperature response curves for final total mass between the two watering treatments. The temperature optimum for the final mass in  $W_{\text{const}}$  ( $25.4 \pm 0.46 \text{ }^\circ\text{C}$ ) was  $\sim 3 \text{ }^\circ\text{C}$  lower than that in  $W_{\text{incr}}$  ( $28.3 \pm 0.36 \text{ }^\circ\text{C}$ ; Figure 3a, Table 2). Also, the peak final mass at  $T_{\text{opt}}$  of  $W_{\text{const}}$  ( $6.54 \pm 0.33 \text{ g}$ ) was significantly lower than that in the  $W_{\text{incr}}$  treatment ( $11.75 \pm 0.61 \text{ g}$ ; Figure 3, Table 2). Plant height, diameter and the estimated total dry mass diverged between the two watering treatments approximately 2 weeks after the implementation of the watering treatments (Figure S2, S3, S4). Plant mass diverged between the two watering temperatures at growth temperatures above  $21.5 \text{ }^\circ\text{C}$  where  $W_{\text{const}}$  showed significantly lower final mass compared to the  $W_{\text{incr}}$  treatment (Figure 3a). The  $\Omega$  parameter of final mass, indicating the breadth of

response relative to the peak at the temperature optimum, was not significantly different between the two watering treatments (Table 2).

Similar to final total plant mass, we observed peaked temperature response curves for three biomass components; leaves, stem and roots (Figure 3b, c, d respectively). The temperature optima of all three components were significantly lower for  $W_{\text{const}}$  compared to the  $W_{\text{incr}}$  (Table 2). For both watering treatments, the temperature optima of leaf and stem mass were similar to the temperature optimum of final total plant mass. However, for root mass, the temperature optimum was approximately 2 °C lower compared to the leaf, stem and total mass for in both watering treatments (see Table 2). Collectively, these results indicate that constrained water inputs with increased growth temperature reduced the temperature optimum of growth to a lower temperature relative to well-watered conditions.

#### *Plant biomass ratios*

We observed similar slopes for two watering treatments ( $\sim 0.6$ ;  $P > 0.05$ ; Table 1) for the regression between leaf mass and total plant biomass (Figure 4a). Hence, the fraction of total biomass found in leaves (leaf mass ratio) did not differ between the two watering treatments after accounting for variation in seedling biomass across treatments. The slope for stem mass vs total plant biomass was significantly lower in  $W_{\text{const}}$  plants ( $0.21 \pm 0.04$ ) compared to the  $W_{\text{incr}}$  ( $0.31 \pm 0.05$ ; Figure 4b, Table 1), indicating a reduced fractional allocation to stem biomass in the water-constrained vs well-watered treatment. In addition, the analysis showed an increased biomass allocation to roots under constrained water inputs, where the slope was higher in  $W_{\text{const}}$  seedlings ( $0.26 \pm 0.05$ ) compared to the  $W_{\text{incr}}$  ( $0.18 \pm 0.04$ ; Figure 4c, Table 1). We infer that the indirect effect of warming on water availability led to an increased allocation to root biomass at the expense of stem.

The total plant leaf area at final harvest showed a peaked response to growth temperature (Figure 5a).  $W_{\text{const}}$  showed an approximately 3 °C lower optimum temperature for total leaf area compared to  $W_{\text{incr}}$  ( $25.6 \pm 0.4$  vs  $28.4 \pm 0.4$  °C). Also, the peak leaf area at the temperature optima was significantly lower in  $W_{\text{const}}$  than  $W_{\text{incr}}$  (Table 2). Mean leaf size also showed a similar peaked pattern with a peak at  $\sim 26$  °C, but the values were not significantly different between the two watering treatments at either lower ( $< 22$  °C) or higher ( $> 30$  °C) growth temperatures (Figure 5b). However, leaf size at the temperature optimum was significantly smaller for  $W_{\text{const}}$  treatment compared to  $W_{\text{incr}}$  treatment (Table 2). We observed a clear difference in the total number of

leaves per plant, whereby  $W_{\text{const}}$  plants had significantly fewer leaves per plant compared to  $W_{\text{incr}}$  at growth temperatures above 25 °C (Figure 5c). Specific leaf area (SLA) showed relatively stable values across the range of  $T_{\text{growth}}$  (Figure 5d). SLA was similar between two watering treatments at both lowest (18 °C) and highest (35.5 °C) growth temperatures, but showed significantly lower SLA values in  $W_{\text{const}}$  plants than the well-watered treatment at other growth temperatures (Figure 5d). Collectively, these results indicate that the indirect effect of warming on water availability did not alter the proportion of plant biomass allocated to leaves, but reduced the total leaf area displayed per plant through a reduction in number of leaves, reduced leaf size and lower SLA.

### Leaf net photosynthesis

The leaf net photosynthesis rates measured at mid-day in-situ  $T_{\text{growth}}$  and saturating PPFD ( $A_{\text{sat}}$ ) showed a peaked response with the measurement leaf temperature across growth temperatures (Figure 6a). The temperature optimum for photosynthesis ( $T_{\text{optA}}$ ) of  $W_{\text{const}}$  treatment ( $27.7 \pm 0.60$  °C) was  $\sim 1.5$  °C lower than the  $W_{\text{incr}}$  ( $29.1 \pm 0.38$  °C), but with overlapping 95% CI (Table 2). Net photosynthesis values at  $T_{\text{optA}}$  ( $A_{\text{opt}}$ ) and the curvature parameter ( $\Omega$ ) were slightly lower in  $W_{\text{const}}$  plants, but the 95 % CIs overlapped. Nonlinear mixed model analysis suggested that the three temperature response parameters ( $T_{\text{optA}}$ ,  $A_{\text{opt}}$  and  $\Omega$ ) were significantly different between watering treatments, although the differences were small (Table 2). We observed a clear divergence in temperature response curves at leaf temperatures above  $T_{\text{optA}}$  where the leaf net photosynthesis ( $A_{\text{sat}}$ ) of the  $W_{\text{const}}$  treatment was significantly lower than the well-watered  $W_{\text{incr}}$  treatment (Figure 6a). In well-watered plants, leaf net photosynthesis increased by 46% between the 18 °C and 21.5 °C growth temperatures and maintained relatively similar assimilation rates up to 28.5 °C, then declined with further increases in growth temperature. The sensitivity was found to be similar for the water-limited plants under constant water inputs at growth temperatures below 21.5 °C, but the photosynthesis rate started to decline at a lower  $T_{\text{growth}}$  ( $\sim 25$  °C) compared to  $W_{\text{incr}}$  treatment. In general, the photosynthetic rates were similar across the watering treatments with the exception of the warmest growth temperature, in which  $A_{\text{sat}}$  of  $W_{\text{const}}$  plants was reduced by 31% compared to the  $W_{\text{incr}}$  plants. We observed a decreasing trend for the apparent maximum carboxylation capacity at 25 °C ( $V_{\text{cmax25}}$ ) with increasing growth temperature (Figure S5). However,  $V_{\text{cmax25}}$  values were similar across the watering treatments at any growth temperature treatment as 95% CIs of the means were overlapping (Figure S5).

Stomatal conductance ( $g_s$ ) did not differ between watering treatments at leaf temperatures below 30 °C (Figure 6b). Overall,  $g_s$  was remarkably high in these young and rapidly growing plants. Plants in both watering treatments exhibited a marked increase in  $g_s$  from leaf temperatures of 18 °C to 25 °C, then gradually decreased in response to further increases in leaf temperature. At high leaf temperatures ( $>35$  °C),  $g_s$  of  $W_{\text{const}}$  plants was significantly lower than the  $W_{\text{incr}}$  plants (Figure 6b). Well-watered plants exhibited increased transpiration rates with higher growth temperatures above 30 °C, and therefore transpiration rates of  $W_{\text{incr}}$  plants were significantly higher than  $W_{\text{const}}$  at higher growth temperatures (Figure 6c). As a result, the difference between leaf temperature and air temperature (inside the Licor 6400XT leaf cuvette) at growth temperatures above 25 °C was always higher for the  $W_{\text{const}}$  plants compared to the  $W_{\text{incr}}$  plants

(Figure S6). Also, there was a clear divergence in the  $C_i:C_a$  ratio between the two watering treatments in that the  $C_i:C_a$  ratio was significantly lower in  $W_{\text{const}}$  plants compared to  $W_{\text{incr}}$  at leaf temperatures above 30 °C (Figure 6d).

#### *Leaf, stem and root respiration*

With increasing growth temperature, we observed an asymptotically decreasing response of mass-based leaf respiration rates ( $R_{L25}$ ) measured at a standard temperature of 25 °C for the two watering treatments (Figure 7a). This reduction in  $R_{L25}$  with increasing  $T_{\text{growth}}$  reflects respiratory acclimation to temperature, as assessed here using the set-temperature method (Atkin et al. 2005; Drake et al. 2016). There was no significant difference in  $R_{L25}$  between watering treatments at  $T_{\text{growth}}$  below 25 °C (overlapping 95% CIs and pooled t-test P value > 0.05). However, at  $T_{\text{growth}}$  above 25 °C,  $W_{\text{const}}$  plants showed significantly lower  $R_{L25}$  values (pooled t-test P value < 0.01) compared to well-watered plants (Figure 7a).

We observed similar asymptotically declining trends for mass-based stem and root dark respiration rates at 25 °C ( $R_{S25}$  and  $R_{R25}$  respectively) with increasing growth temperature (Figure 7b, c). In both watering treatments, respiration rates at 25 °C, steeply decreased from the coldest; 18 °C  $T_{\text{growth}}$  to 25 °C and remained approximately invariant with increasing  $T_{\text{growth}}$  above 25 °C. Hence, the data showed acclimation of respiration to growth temperatures below 25 °C and constrained acclimation at growth temperatures above 25 °C. Both stem and root respiration rates did not differ between the water treatments, exhibiting overlapping 95% CIs. These results provide evidence that constrained water inputs reduced whole plant respiration at higher growth temperatures mainly due to decreased leaf respiration rates. However, the lack of acclimation evident at temperatures exceeding 25 °C indicates that whole-plant respiration rates increased as growth temperatures continued to increase.

## Discussion

In this study, we experimentally separated the direct effect of temperature *per se* from the indirect effect of temperature on water availability on the temperature response of tree growth and physiology. The direct effect of temperature on growth was substantial, but showed a peaked response: growth increased up to 28.3 °C and decreased thereafter. The indirect effect of temperature via water limitation was also large. We found that water limitation resulted in lower temperature optima for both photosynthesis and growth compared to well-watered conditions. Thus, direct effects and indirect effects were both important. Remarkably, the effect of constraining water inputs was much stronger on growth than on photosynthesis, with growth rates being approximately halved at higher temperatures. Detailed physiological measurements enabled us to investigate the mechanisms underlying these responses.

### *Photosynthesis*

Our results suggested that, at higher growth temperatures, photosynthesis rates were low when plants are grown under constant water inputs instead of water inputs that increased to match plant demand. The apparent  $V_{\text{cmax}25}$  values were remarkably similar between the two watering treatments, but the ratio of CO<sub>2</sub> concentration inside the leaf air spaces relative to the atmosphere ( $C_i:C_a$ ) was significantly lower under constant water inputs. This analysis indicates a strong stomatal limitation of photosynthesis with increasing growth temperature under constant water input conditions (Drake *et al.*, 2017a, Reich *et al.*, 2018). Our results contrast with several studies that provided evidence for a decrease in photosynthetic capacities under constrained soil moisture conditions (Reich *et al.*, 2018, Xu & Baldocchi, 2003). In well-watered soils, plants show increases in net photosynthetic rate within a broader growth temperature domain because of increasing carboxylation capacity with temperature (Drake *et al.*, 2017b, Gunderson *et al.*, 2009). An increased demand for carbon due to higher growth rates may also play a role in increased photosynthesis (Körner, 2003). However, under low soil moisture conditions, increased stomatal limitation eliminated most of the potential beneficial effects of increasing temperature on photosynthesis. Additionally, plant growth rate is lower under constrained soil moisture, therefore the demand for carbon is less.

Collectively, we suggest, from several lines of evidence, that stomatal conductance drove the observed lower photosynthetic rates and the lower temperature optima for photosynthesis (Lin *et al.*, 2012) when plants are grown under constant water inputs with increasing growth

temperature. A number of studies report reduced photosynthesis in response to warming under constrained water inputs (Duan *et al.*, 2013, Duan *et al.*, 2014, Wertin *et al.*, 2012). However, the responses observed in these studies cannot be directly compared with our results as the direction of the warming effects depends on the reference growth temperature used in the study, as well as the nature of water limitation. In our unique design, the magnitude of water limitation increased with increasing temperature, since the amount of water added was held constant across growth temperatures. In many of the previous studies, the interactive effects of warming and soil water availability on plant growth has been studied in factorial experiments by inducing acute water stress, either by withholding water until plant death (Adams *et al.*, 2009, Duan *et al.*, 2013, Duan *et al.*, 2014) or by adding a pre-determined and reduced amount of water (Gauthier *et al.*, 2014). Nevertheless, the observed photosynthetic responses in this study are strongly supported by the results of a recent field-based direct air and soil warming experiment (Reich *et al.*, 2018) which demonstrated that the magnitude of photosynthetic enhancement that results from experimental warming depends on soil moisture status. Several other studies provided evidence for negative growth responses to temperature due to low soil moisture status (Lazarus *et al.*, 2018, Moyes *et al.*, 2013, Walker & Johnstone, 2014, Wertin *et al.*, 2012, Wertin. *et al.*, 2010). However, it is rare for the direct and indirect effects of warming to be separated in field-based experiments.

Our data showed a larger impact of water limitation on growth than on leaf net photosynthesis at higher temperatures. At growth temperatures above the optimum, photosynthesis decreased approximately by 30%, but the final dry mass decreased by nearly 50%. Further, the temperature optimum for growth decreased by 3 °C under constant water inputs, but the optimum temperature for photosynthesis decreased only by 1.5 °C. Several studies provide evidence for the maintenance of photosynthesis under water deficit (Bogeat-Triboulot *et al.*, 2007, Quick *et al.*, 1992). Collectively, these results suggested that growth is more sensitive to water limitation than photosynthesis (Müller *et al.*, 2011) and leaf net photosynthesis alone is insufficient to explain the reduction in growth (Campany *et al.*, 2017) in response to warming under constrained water inputs.

### *Respiration*

We found that leaf respiration rates (at a set temperature 25 °C;  $R_{L25}$ ) diverged with plant water availability at higher growth temperatures. The observed decrease in  $R_{L25}$  due to water limitation was consistent with findings for other plant species including eucalypts (Ayub *et al.*,

2011, Callister & Adams, 2006, Crous *et al.*, 2012, Crous *et al.*, 2011, Galmés *et al.*, 2007, Huang & Fu, 2000) which reported a decrease in leaf respiration during water stress. At growth temperatures below 28 °C,  $R_{L25}$  was not significantly different between the two watering treatments, likely because the indirect effect of temperature on water availability is smaller at these temperatures. The decrease in leaf respiration rates under drought conditions could be due to decreased substrate availability, or decreased demand for ATP and other respiratory products such as NADH, TCA cycle intermediates (Atkin & Macherel, 2009, Crous *et al.*, 2011). Previous studies with tree species suggest that decreases in leaf respiration rates with water limitation in dry soils are more likely to be due to decreases in the demand for respiratory products, rather than a decrease in substrate supply for respiration (Ayub *et al.*, 2011, Crous *et al.*, 2011). Duan *et al.* (2014) also showed relatively stable whole-plant non-structural carbohydrate concentration (TNC) in response to warming under drought conditions. Hence, we suggest that the observed decline in leaf respiration rates due to constant water inputs in this study could potentially be explained by the reduced demand for respiratory products through decreased leaf growth rates.

Remarkably, stem and root respiration rates did not differ between the two watering treatments. Studies on how stem and root respiration are affected by warming under differential water regimes are uncommon in the literature. Several studies report decreased root respiration rates in response to decreased soil moisture (Bryla *et al.*, 2001, Burton *et al.*, 1998, Huang & Fu, 2000, Jarvi & Burton, 2018), but others suggest that root respiration is not significantly affected by combined effect of warming and reduced water availability (Bryla *et al.*, 1997). However, such comparisons are rare for stem respiration. The lack of change in stem and root respiration rates under constrained water inputs could potentially be explained by the level of stem and root activity during the seedling growth. In reduced soil moisture conditions, the activity of plant roots is perhaps increased in order to extract water and nutrients; therefore, the root energy requirement would increase under water limited conditions, which could be achieved through maintaining root respiration rates. Similarly, maintenance of water transport from roots to canopy would be expected to compensate for the increasing transpiration demand; therefore, the energy demand for stem activity would continue to be high, maintaining relatively stable stem tissue respiration rates (Lambers *et al.*, 2008).

Our results suggest a nearly homeostatic acclimation of leaf, stem and root respiration in response to warming from low to mid temperatures (18-25 °C). However, at growth temperatures above 25 °C, tissue-specific respiration rates markedly increased, indicating a lack of homeostasis.

Our results were comparable with previous studies which reported similar acclimation responses of leaf respiration rates to experimental warming under well-watered conditions (Aspinwall *et al.*, 2016, Drake *et al.*, 2016). The lack of temperature acclimation of respiration at growth temperatures exceeding 25 °C indicates increased whole plant respiration above 25 °C, indicating increased maintenance costs at higher temperatures, owing to reduced growth rates at higher temperatures. Remarkably, these temperature acclimation responses were similar between the two watering treatments. The lack of homeostasis in whole plant respiration (Drake *et al.*, 2017) partly explains the reduction in growth above the optimum. However, it does not explain why growth is so strongly impacted by the water treatment, as our data showed a decrease in whole plant respiration above the optimum under both water treatments.

#### *Allocation patterns and growth*

Why is growth so substantially affected under constrained water inputs when photosynthesis is less affected and whole plant respiration decreases? Our results indicate that incremental warming of growth temperatures under constant water inputs and thus decreasing plant water availability does not alter the proportion of plant biomass allocation to leaves compared to well-watered controls. However, water limitation reduces the total leaf area display of trees by decreasing the total number of leaves produced and reducing their average size. Also, SLA was significantly lower under constant water inputs compared to the well-watered treatment, indicating a reduced amount of photosynthesizing surface area per unit leaf dry mass. Ghannoum *et al.* (2010) reported decreased leaf size due to a direct effect of increasing temperature, but under well-watered conditions. Cunningham and Read (2003) suggested that growth largely depends on the dry matter allocation to plant's productive and supportive tissues; hence, maximum growth is achieved when the allocation to photosynthetic tissues is maximized. Supporting this hypothesis, Xiong *et al.*, (2000) reported improved growth responses to temperature in two Antarctic vascular plant species where, greater growth rates were achieved through greater dry mass allocation to leaves. Decreases in SLA in response to water limitation have previously been observed (Marron *et al.*, 2003, Nautiyal *et al.*, 2002). Evidence from other studies suggests that the accumulation of soluble sugars and phenolic compounds in leaves increases under water stress, which may facilitate maintenance of high turgor pressure in leaves (Marron *et al.*, 2002, Marron *et al.*, 2003). Also, water stress promotes production of thicker cell walls as a strategy to improve cell resistance to collapse and elicit changes in tissue elasticity (Laureano *et al.*, 2008, Niinemets, 2001). Such

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modifications in leaf structure ultimately decrease the amount of leaf area display for photosynthesis per unit leaf dry mass. We found that temperature optimum for final harvest plant mass in both the  $W_{\text{const}}$  and  $W_{\text{incr}}$  treatments mirrored the temperature optima of total plant leaf area, total leaf count and mean leaf size. Taken together, this pattern supports the hypothesis that plants maximize their growth rate and biomass accumulation when C allocation to leaf area display is maximized. Hence, we suggest that the growth is largely affected under constrained water inputs due to the decrease in daily total carbon gain via the cumulative effect of lower photosynthetic rates and decreased SLA and greater proportional respiratory losses at the whole plant level.

### *Implications*

In summary, our results strongly supported our hypothesis that the temperature optima for photosynthesis and growth would be significantly decreased by the indirect effect of increasing water limitation. We demonstrate that a decrease in leaf net photosynthesis, changes in biomass allocation patterns, reduced total leaf area display and lower specific leaf area are the key underlying mechanisms by which this reduction in temperature optima of growth occurs. Our work highlights that there is an important indirect effect of warming on water supply to trees, increasing water limitation to growth even where rainfall is unchanged. Our study species, *E. tereticornis*, is distributed along the east coast of Australia where, for the most part, annual potential evapotranspiration exceeds mean annual rainfall. Current projections for future climate in this region under RCP8.5 indicate a warming of mean annual temperature above the climate of 1986-2005 by 2.8 to 5.1 °C by 2090. However, rainfall projections remain unclear, with a large degree of uncertainty (CSIRO & BOM, 2019). Our work indicates that even if mean annual precipitation does not change, water limitation of growth will increase with the projected climate warming. Experiments that do not consider this effect will clearly overestimate the positive impacts of warming on growth. We emphasise the need to consider this indirect effect in experiments, and in terrestrial biosphere models attempting to predict how forests will fare under climate warming in the future.

## **Acknowledgement**

This research was supported by an Australian Research Council Discovery Project (DP140103415) with additional support from the Hawkesbury Institute for the Environment and Western Sydney University. DK was supported by a Western Sydney University international PhD scholarship. We thank Gavin Mckenzie, Goran Lopaticki and Burhan Amiji for technical assistance.

## **Author contribution statement**

Project conceived by BEM. Data collection and analyses designed and carried out by DPK with guidance from BEM and JED. Manuscript writing led by DK, BEM and JED. MGT contributed to data collection and made a substantial contribution to the experimental design, data interpretation, and writing. All co-authors contributed data, ideas, and edited the manuscript.

## **Data Sharing and Data Accessibility**

The dataset used for this study is publicly available (Drake et al., 2016b) and the analysis code to reproduce all the results, including the figures and tables, is available as a *git* repository (<https://bitbucket.org/Kumarathunge/great-drought>).

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## Tables

Table 1. Results of the standardized major axis regression of the predawn and mid-day water potentials and allometric relationships of leaf, stem and root mass fractions by watering treatments (data shown Figure 2 and 4 in the main text). Treatments; warming with water inputs increased to match plant demand ( $W_{incr}$ ) and warming with constant water inputs ( $W_{const}$ ). P-values < 0.05 depict significantly different slopes between treatments for a given regression. Values in parentheses are 95% confidence intervals of estimates.

Regression	Treatment	Intercept	Slope	$r^2$	P-value
$\Psi_{pd}$ (MPa) vs $T_{growth}$	$W_{incr}$	0.24 (-0.1 - 0.5)	-0.02 ( -0.04 - -0.02)	0.90	0.0468
	$W_{const}$	0.45 (0.1 - 0.8)	-0.04 ( -0.05 - -0.03)	0.94	
$\Psi_{md}$ (MPa) vs $T_{growth}$	$W_{incr}$	-0.05 (-0.8 - 0.7)	-0.05 ( -0.09 - -0.03)	0.85	0.3170
	$W_{const}$	0.08 (-0.9 - 1.1)	-0.07 ( -0.12 - -0.04)	0.87	
Leaf mass (g) vs total mass (g)	$W_{incr}$	-0.31 (-0.7 - 0.1)	0.6 (0.55 - 0.65)	0.95	0.1479
	$W_{const}$	-0.35 (-0.6 - -0.1)	0.65 (0.59 - 0.71)	0.96	
Stem mass (g) vs total mass (g)	$W_{incr}$	-0.21 (-0.7 - 0.2)	0.31 (0.26 - 0.36)	0.55	0.0195
	$W_{const}$	0.10 (-0.2 - 0.4)	0.21 (0.16 - 0.28)	0.80	
Root mass (g) vs total mass (g)	$W_{incr}$	-0.03 (-0.4 - 0.3)	0.18 (0.14 - 0.22)	0.62	0.0131
	$W_{const}$	-0.37 (-0.7 - -0.1)	0.27 (0.21 - 0.34)	0.68	

Table 2. Parameters of the temperature response of growth and photosynthesis (Eqn 2). Treatments; warming with water inputs increased to match plant demand ( $W_{incr}$ ) and warming with constant water inputs ( $W_{const}$ ). Statistically significant differences in parameters between treatments are indicated by different letters. Values in parentheses are 95% confidence intervals of estimates.

Variable	Treatment	$T_{opt}$ (°C)	$k(T_{opt})$	$\Omega$ (°C)
Final total mass (g)	$W_{incr}$	28.3 (27.5-29.0) <sup>a</sup>	11.7 (10.5-13.0) <sup>a</sup>	8.3 (7.1-9.5) <sup>a</sup>
	$W_{const}$	25.4 (24.5-26.4) <sup>b</sup>	6.5 (5.9-7.2) <sup>b</sup>	10.4 (8.6-12.2) <sup>a</sup>
Final leaf mass (g)	$W_{incr}$	28.4 (27.5-29.3) <sup>a</sup>	6.6 (5.7-7.4) <sup>a</sup>	8.2 (6.7-9.7) <sup>a</sup>
	$W_{const}$	25.6 (24.8-26.5) <sup>b</sup>	3.9 (3.5-4.4) <sup>b</sup>	9.4 (7.9-10.9) <sup>a</sup>
Final stem mass (g)	$W_{incr}$	29.5 (28.3-30.7) <sup>a</sup>	3.21 (2.7-3.7) <sup>a</sup>	9.0 (7.0-10.9) <sup>a</sup>
	$W_{const}$	27.4 (25.5-29.2) <sup>b</sup>	1.4 (1.2-1.5) <sup>b</sup>	13.5 (8.9-18.1) <sup>b</sup>
Final root mass (g)	$W_{incr}$	26.3 (25.7-26.9) <sup>a</sup>	2.1 (1.9-2.3) <sup>a</sup>	7.3 (6.4-8.2) <sup>a</sup>
	$W_{const}$	23.3 (21.6-25.0) <sup>b</sup>	1.3 (1.1-1.5) <sup>b</sup>	10.3 (7.7-13.1) <sup>b</sup>
$A_{sat}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$W_{incr}$	29.1 (28.3-29.8) <sup>a</sup>	25.5 (24.3-26.8) <sup>a</sup>	15.3 (13.7-17.6) <sup>a</sup>
	$W_{const}$	27.7 (26.3-28.8) <sup>b</sup>	23.9 (22.1-25.6) <sup>b</sup>	14.1 (12.1-17.1) <sup>a</sup>
Leaf area ( $\text{cm}^2$ )	$W_{incr}$	28.0 (27.38-28.68) <sup>a</sup>	1310 (1180-1440) <sup>a</sup>	7.8 (6.8-8.8) <sup>a</sup>
	$W_{const}$	25.0 (23.83-26.08) <sup>b</sup>	584 (515-654) <sup>b</sup>	10.3 (8.2-12.4) <sup>b</sup>
Mean leaf size ( $\text{cm}^2$ )	$W_{incr}$	26.1 (24.83-27.33) <sup>a</sup>	31.0 (25.8-36.2) <sup>a</sup>	9.0 (6.8-11.1) <sup>a</sup>
	$W_{const}$	25.6 (24.0-27.1) <sup>a</sup>	21.9 (19.4-24.4) <sup>b</sup>	14.2 (10.0-18.4) <sup>a</sup>
Leaf count (#)	$W_{incr}$	31.1 (28.1-34.2) <sup>a</sup>	53.8 (45.2-62.3) <sup>a</sup>	12.3 (7.8-17.3) <sup>a</sup>
	$W_{const}$	25.0 (22.6-27.4) <sup>b</sup>	27.3 (23.6-31.0) <sup>b</sup>	15.2 (9.1-21.9) <sup>b</sup>

## Figure legends

**Figure 1.** Daily mean soil volumetric water content ( $\theta$ ) of plants at six growth temperatures; (a) 18°C – (f) 35.5°C. The ticks along the x-axis denote the date that leaf-level gas exchange was measured (left) and the final harvest was completed (right). The shaded horizontal line in each panel depicts the  $\theta$  value at 70% of the field capacity. Legend in panel (a) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).

**Figure 2.** Mean pre-dawn (a) and mid-day (b) leaf water potential at different growth temperatures ( $n=5$ ) at the end of the study. Error bars depict 95% CI of the mean. Legend in panel (a) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).

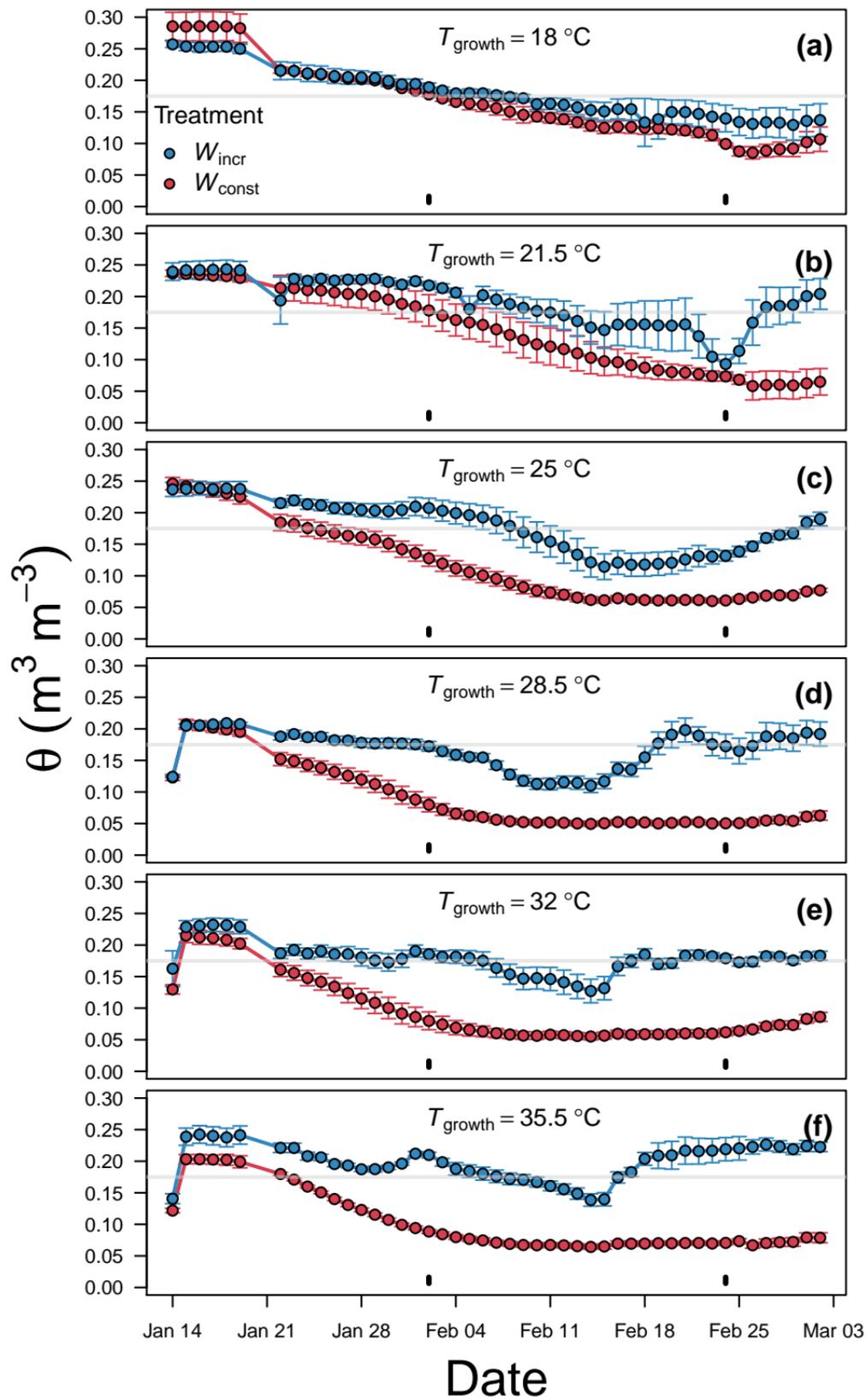
**Figure 3.** Temperature response of final total plant mass (45-day period) (a) and its components leaf mass (b), stem mass (c) and root mass (d). Growth  $T_{\text{air}}$  is the daily mean air temperature across the experiment. Lines depict the fitted temperature response function (Eqn2) and the shaded areas depict the 95% CI of the predictions. Filled circles are the measured data and the error bars depict  $\pm 1\text{SE}$  ( $n=5$ ). Legend in panel (a) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).

**Figure 4.** Fraction of total plant biomass allocated to (a) leaves, (b) stem and (c) roots. Lines represent standardized major axis fitting of the allometric relationships of mass fraction by water treatment. Filled circles are the measured data at the final harvest across all growth temperatures. Note the slopes in panel (a) are not significantly different and in panels (b) and (c) are significantly different between water treatments (Table 1). Legend in panel (c) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).

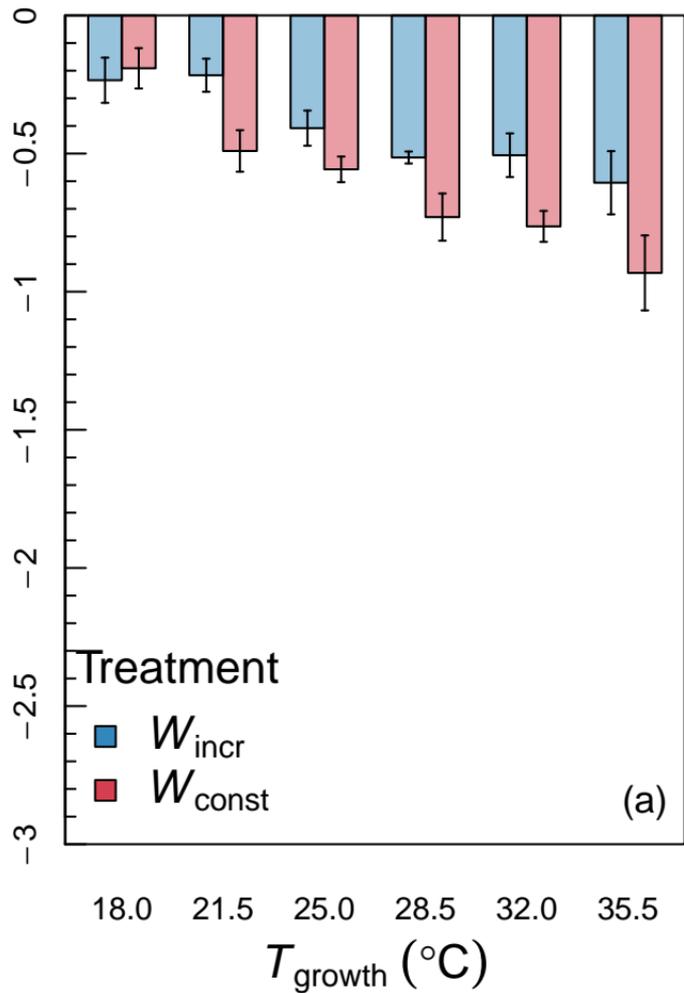
**Figure 5.** Temperature response of mean total leaf area (a) mean individual leaf size (b) total number of leaves per plant (c) and specific leaf area (d; SLA) measured at final harvest after 45 days. Lines depict the fitted temperature response function (Eqn 2) in panels a-d and simple linear regression fits in panel e. Shaded areas depict the 95% CI of the predictions. Error bars depict  $\pm 1\text{SE}$  ( $n = 5$ ). Legend in panel (a) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).

**Figure 6.** Temperature response of leaf net photosynthesis ( $A_{\text{sat}}$ ) (a), stomatal conductance ( $g_s$ ) (b), leaf transpiration ( $E$ ) (c) and  $[\text{CO}_2]$  ratio of intercellular: ambient air ( $C_i:C_a$ ) (d) of two treatments. Data measured at a PPFD of  $1500 \mu\text{mol m}^{-2}\text{s}^{-1}$  and at the mid-day in-situ growth temperatures. Lines depict the fitted temperature response function (Eqn1) in panel (a) and fitted general additive models in panel (b-d). The shaded areas depict the 95% CI of the predictions. Filled circles are the measured data and the error bars depict  $\pm 1\text{SE}$  ( $n = 8$ ). Legend in panel (d) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).

**Figure 7.** Temperature response of mass based respiration rates of leaf (a), stem (b) and root (c) measured at a standard temperature of 25°C. Lines depict the fitted general additive models and the shaded areas depict the 95% CI of the predictions. Filled circles are the measured data. Error bars depict  $\pm 1\text{SE}$  ( $n = 5$ ). In panel (a), symbol \* depicts significant differences ( $\alpha=0.05$ ) between watering treatments at a given Growth  $T_{\text{air}}$ . Legend in panel (a) depicts two treatments; water inputs increasing with temperature to match plant demand at all temperatures ( $W_{\text{incr}}$ ) and water inputs constant for all temperatures, matching demand for coolest grown plants in the 18 °C treatment ( $W_{\text{const}}$ ).



Leaf  $\Psi_{pd}$  (MPa)



Leaf  $\Psi_{md}$  (MPa)

