

No evidence of homeostatic regulation of leaf temperature in *Eucalyptus parramattensis* trees: integration of CO₂ flux and oxygen isotope methodologies

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Summary

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- Thermoregulation of leaf temperature (T_{leaf}) may foster metabolic homeostasis in plants, but the degree to which T_{leaf} is moderated, and under what environmental contexts, is a topic of debate. Isotopic studies inferred the temperature of photosynthetic carbon assimilation to be a constant value of c. 20°C; by contrast, leaf biophysical theory suggests a strong dependence of T_{leaf} on environmental drivers. Can this apparent disparity be reconciled?

- We continuously measured T_{leaf} and whole-crown net CO₂ uptake for *Eucalyptus parramattensis* trees growing in field conditions in whole-tree chambers under ambient and +3°C warming conditions, and calculated assimilation-weighted leaf temperature (T_{L-AW}) across 265 d, varying in air temperature (T_{air}) from –1 to 45°C. We compared these data to T_{L-AW} derived from wood cellulose δ¹⁸O.

- T_{leaf} exhibited substantial variation driven by T_{air} , light intensity, and vapor pressure deficit, and T_{leaf} was strongly linearly correlated with T_{air} with a slope of c. 1.0. T_{L-AW} values calculated from cellulose δ¹⁸O vs crown fluxes were remarkably consistent; both varied seasonally and in response to the warming treatment, tracking variation in T_{air} .

- The leaves studied here were nearly poikilothermic, with no evidence of thermoregulation of T_{leaf} towards a homeostatic value. Importantly, this work supports the use of cellulose δ¹⁸O to infer T_{L-AW} , but does not support the concept of strong homeothermic regulation of T_{leaf} .

Introduction

Temperature directly affects the physiological activity of organisms (Gillooly *et al.*, 2001; Atkin & Tjoelker, 2003; Clarke & Fraser, 2004; Sage & Kubien, 2007). Plants are sessile, typically produce thin leaves with low heat capacity, and do not produce substantial metabolic heat (but see some exceptions; Herrera & Pozo, 2010; Zhu *et al.*, 2011; Leigh *et al.*, 2012). As such, leaf temperature (T_{leaf}) is strongly influenced by environmental variation in air temperature (T_{air}), solar radiation, wind speed, and humidity. There is a well-developed literature regarding the prediction of T_{leaf} from biophysical equations (Gates, 1965; Wang & Leuning, 1998; Campbell & Norman, 2000; Jones, 2013) that are widely implemented in ecosystem and earth system models (Leuning *et al.*, 1995; Sellers *et al.*, 1996; Baldocchi *et al.*, 2002; Ryder *et al.*, 2016; Dong *et al.*, 2017).

Several aspects of plant form and function influence T_{leaf} values relative to T_{air} . The structure of plants alters the turbulence of the atmosphere, the interception and scattering of light

through plant canopies, and the radiation loads on leaves and soil, all of which influence T_{leaf} (Gates, 1965; Baldocchi & Meyers, 1998; Albertson *et al.*, 2001; Smith & Hughes, 2009; Vogel, 2009; Dong *et al.*, 2017). Arctic and alpine plants can have T_{leaf} values that are considerably higher than T_{air} during sunlit periods of the summer, as closely-spaced leaves situated close to the ground can absorb large amounts of solar radiation with low rates of heat return to the atmosphere (Salisbury & Spomer, 1964; Hadley & Smith, 1987). Narrow leaves tend to be well-coupled to the atmosphere and have little T_{leaf} to T_{air} difference, while wide leaves can be much cooler or warmer than T_{air} depending on stomatal conductance and latent cooling (Smith, 1978; Jarvis & McNaughton, 1986; Yates *et al.*, 2010; Wright *et al.*, 2017). Leaf inclination, clumping, thickness, size, and canopy position also affect T_{leaf} relative to T_{air} (Medina *et al.*, 1978; Ball *et al.*, 1988; Smith & Carter, 1988; Leuzinger & Körner, 2007; Leigh *et al.*, 2012; Wright *et al.*, 2017). These structural influences on T_{leaf} have been described as passive thermoregulation (Michaletz *et al.*, 2015). In general, the thermodynamics of sensible and

latent heat fluxes are such that net radiation tends to increase T_{leaf} relative to T_{air} at low T_{air} , while latent cooling tends to dominate at higher temperatures, such that T_{leaf} to T_{air} difference tends to decline with increasing T_{air} (Penman, 1948; Gates, 1965; Monteith, 1965; Campbell & Norman, 2000; Dong *et al.*, 2017). Transpirational cooling of T_{leaf} appears to be a mechanism of avoiding exceptionally hot leaf temperatures during heat waves, at least in some species (Upchurch & Mahan, 1988; Drake *et al.*, 2018; Guha *et al.*, 2018). This cooling mechanism likely depends on water availability and hydraulic conductivity under extreme conditions.

There has been considerable recent interest and controversy regarding thermoregulation of T_{leaf} (Helliker & Richter, 2008; Michaletz *et al.*, 2015; Chu *et al.*, 2016; Dong *et al.*, 2017). Terminology has been an issue in this field, and we recognize that a diversity of terms have been used to describe deviation between T_{leaf} and T_{air} (Linacre, 1964; Gates, 1965; Leuzinger & Körner, 2007; Michaletz *et al.*, 2015). Here, we use the term ‘thermoregulation’ to describe the systematic deviation of T_{leaf} from T_{air} , as this term is general and does not imply a specific directionality. We do not intend ‘thermoregulation’ to invoke a teleological meaning, however. For example, the surface temperatures of manufactured materials such as brick and concrete differ substantially from T_{air} in a manner affected by solar radiation and the thermal characteristics of the materials themselves (Liu *et al.*, 2017), but this does not involve intrinsic regulatory mechanisms. However, systematic deviation of T_{leaf} from T_{air} could strongly impact leaf physiology, whether this arises from an actively regulated physiological mechanism such as stomatal control, or a passive mechanism driven by the structure of leaves and crowns.

Some of the recent interest in leaf thermoregulation began with Helliker & Richter (2008), who utilized $\delta^{18}\text{O}$ measurements of tree wood cellulose and asserted that the leaf temperature of carbon (C) assimilation was a constant value of $21.4 \pm 2.2^\circ\text{C}$ across a latitudinal dataset spanning *c.* 35°C in mean annual temperature (MAT). In brief, this method uses $\delta^{18}\text{O}$ measurements to calculate the vapor pressure inside the leaf airspaces and calculates T_{leaf} assuming that leaf airspaces are fully hydrated. This work has been influential in the field of plant thermal biology and has contributed to a more general framework regarding relationships between plant traits, thermoregulation, and primary production (Michaletz *et al.*, 2014, 2015; Chu *et al.*, 2016). Importantly, Helliker & Richter (2008) did not argue that T_{leaf} was always a constant value of *c.* 20°C for all leaves. They argued that the T_{leaf} that was recorded into the wood cellulose $\delta^{18}\text{O}$ record was *c.* 20°C , recognizing that $\delta^{18}\text{O}$ values are inherently weighted towards periods of maximal CO₂ assimilation, and thus record an assimilation-weighted T_{leaf} ($T_{\text{L-AW}}$). That is, the $T_{\text{L-AW}}$ of *c.* 20°C observed by Helliker & Richter (2008) could arise from a T_{leaf} that varied with the environment but was differentially ‘sampled’ by photosynthesis at values that averaged *c.* 20°C . Determining the physiological and biophysical influences on $T_{\text{L-AW}}$ would be helpful in validating the use of cellulose $\delta^{18}\text{O}$ observations to reconstruct T_{leaf} from tree rings, to quantify the degree to which $T_{\text{L-AW}}$ and T_{leaf} differs from T_{air} in plants, and to establish the nature of this difference.

The claim of a constant $T_{\text{L-AW}}$ spurred further research, particularly in the interpretation of tree-ring cellulose $\delta^{18}\text{O}$ data (Sternberg, 2009; Kruse *et al.*, 2012; Gessler *et al.*, 2014) and the assumptions underlying the calculation of leaf temperatures from cellulose $\delta^{18}\text{O}$ (Sternberg & Ellsworth, 2011; Xiao *et al.*, 2012; Griffis, 2013; Song *et al.*, 2014; Helliker *et al.*, 2018). Subsequent observations of wood cellulose $\delta^{18}\text{O}$ offered independent support for the concept of an approximately constant $T_{\text{L-AW}}$ of *c.* 20°C in boreal, temperate, and subtropical forests, but not in tropical forests (Song *et al.*, 2011; Helliker *et al.*, 2018). Thus the $\delta^{18}\text{O}$ measurements and interpretations seem to be robust. How can we reconcile this literature suggesting a constant $T_{\text{L-AW}}$ with the well-established biophysical understanding of a highly variable T_{leaf} that responds to the environment?

It is important to distinguish between thermoregulation of T_{leaf} *per se* from the concept of constant $T_{\text{L-AW}}$ (Helliker & Richter, 2008). If leaves were perfectly poikilothermic and equilibrated with T_{air} with no time lag, T_{leaf} would be equivalent to T_{air} (Fig. 1, 1 : 1 line). By contrast, if leaves were completely homeothermic, T_{leaf} would be invariant with T_{air} (Fig. 1, flat dotted line). Direct measurements of T_{leaf} tend to be intermediate (Fig. 1, dashed line), which has been previously described as ‘limited homeothermy’ (Michaletz *et al.*, 2016). A key assertion of a constant $T_{\text{L-AW}}$ (Helliker & Richter, 2008) is that the temperature dependence of photosynthesis differentially weights the temperatures recorded into the tree ring $\delta^{18}\text{O}$ values. During cold days, photosynthesis would predominate during the warm afternoons, such that $T_{\text{L-AW}}$ would exceed the average daytime T_{air} . Conversely, on hot days, photosynthesis would be highest during the relatively cool conditions in the morning, such that $T_{\text{L-AW}}$ would be less than the average daytime T_{air} . There have been few direct tests of this idea, apart from a recent eddy-covariance synthesis which found support for a constant $T_{\text{L-AW}}$ across forest ecosystems (Helliker *et al.*, 2018).

In this study, we apply the unique infrastructure of whole-tree chambers to continuously document leaf temperatures and whole-tree photosynthetic CO₂ assimilation, enabling a direct and independent calculation of $T_{\text{L-AW}}$. We also utilize seasonal measurements of wood cellulose $\delta^{18}\text{O}$ to calculate $T_{\text{L-AW}}$ as in Helliker & Richter (2008). We complement seasonal and day-to-day variation in T_{air} with experimental warming and heatwave treatments to robustly explore T_{leaf} under a wide range of environmental conditions. Based on the fundamental hypotheses that T_{leaf} is moderated relative to T_{air} and that photosynthesis has a thermal dependence, we predict that T_{leaf} will be correlated with T_{air} with a slope between 1.0 and zero, and that $T_{\text{L-AW}}$ will have no significant correlation with T_{air} (Fig. 1).

Materials and Methods

Site description

This experiment utilized 12 whole-tree chambers (WTCs) in Richmond, New South Wales (Australia; lat $33^\circ 36' 40''\text{S}$, long $150^\circ 44' 26.5''\text{E}$). This infrastructure was extensively described by Barton *et al.* (2010). An aluminum frame is covered with a

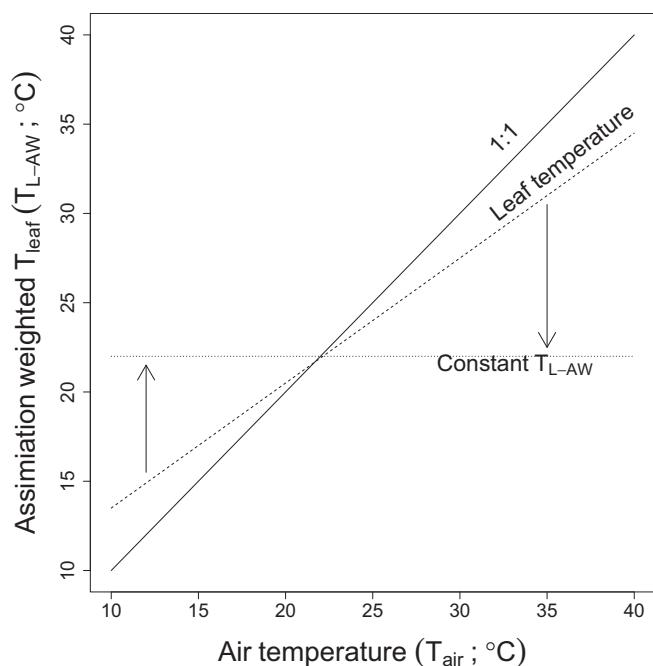


Fig. 1 Conceptual depiction of the relationship between air temperature (T_{air}), and assimilation-weighted T_{leaf} ($T_{\text{L-AW}}$). If there is no thermoregulation of T_{leaf} , then T_{leaf} will follow T_{air} with a slope of 1.0. If there is partial thermoregulation of T_{leaf} that tends towards a homeostatic T_{leaf} , T_{leaf} will still be strongly correlated with T_{air} , but with a slope that is less than 1.0 (dashed line: partial thermoregulation of T_{leaf}). If T_{leaf} thermoregulation is complete, then T_{leaf} will be nearly constant despite variation in T_{air} (dotted line: complete thermoregulation). Note that the slope of assimilation-weighted T_{leaf} vs T_{air} may be different than the slope of T_{leaf} vs T_{air} , particularly if photosynthesis occurs at different rates at different temperatures (arrows).

transparent, self-cleaning, ultra-thin ethylene-tetrafluoroethylene (ETFE) copolymer film. Temperature and humidity were dynamically controlled within each chamber by circulating the chamber air through an air handling unit, which contained a cold condensing coil and electric heaters. The volumetric airflow through the air handling units was approximately constant with an average value of c. $3 \text{ m}^3 \text{ s}^{-1}$, leading to an average estimated windspeed in the chambers of c. 0.3 m s^{-1} . The mean annual temperature at this location was 17°C , the mean maximum temperature of the warmest month (January) was 30°C , and the mean minimum temperature of the coldest month was 3.6°C .

The WTCs enclosed individual trees rooted in soil inside large cylindrical structures (3.25 m in diameter, 9 m in height, volume of c. 53 m^3). The WTCs continuously measured the net exchange of CO_2 and H_2O between entire tree crowns and their surrounding atmosphere while controlling atmospheric CO_2 concentrations, T_{air} , and atmospheric humidity (Barton *et al.*, 2010, 2012; Drake *et al.*, 2016). T_{air} was measured within each tree crown with duplicate, shielded, and aspirated thermistors (Dwyer RHP-2d2B, Michigan City, IN, USA and Mamac TE-702-A-12-A, Minneapolis, MN, USA). The T_{air} within the ambient temperature chambers was dynamically controlled to match the T_{air} measured outside the chambers by another set of duplicate, shielded, and aspirated thermistors. The rooting volume of each tree was compartmentalized with a root

exclusion barrier that extended vertically to 1-m depth, although some trees may have accessed water deeper in the soil profile (Drake *et al.*, 2018).

Seeds of *Eucalyptus parramattensis* were acquired from Harvest Seeds and Native Plants (Terry Hills, NSW, Australia) and germinated in a local shade house. Three months after seed germination, six potted seedlings were placed into each WTC, and the experimental warming treatment (see below, ‘Experimental description’ section) was initiated on 28 Oct 2015. One seedling was planted into the soil within each WTC on 23 Dec 2015; at that time average seedling height was 60 cm. Leaves of these *E. parramattensis* trees were evergreen, pendulous and lanceolate with a leaf width of c. 3 cm and an average leaf size of 32 cm^2 (SD of 13 cm^2 , $n = 420$ leaves), which is average to moderately large compared to the global distribution of plant leaf area at this latitude (Wright *et al.*, 2017).

Experimental description

We implemented a warming experiment beginning on 28 Oct 2015. Six chambers tracked the natural variation in T_{air} and relative humidity (RH) observed at the site (the ‘ambient’ treatment). Six chambers tracked the ambient T_{air} with $+3^\circ\text{C}$ warming while also tracking the ambient RH (the ‘warmed’ treatment). The warming treatment was applied to both the aboveground and belowground compartments. The average warming was $+2.9^\circ\text{C}$ ($\pm \text{SD}$ of 0.6 across 265 d) for T_{air} in the crown compartment, $+2.9^\circ\text{C}$ (± 0.8) for soil temperature (T_{soil}) at 5-cm depth, $+3.0^\circ\text{C}$ (± 0.5) for T_{soil} at 10-cm depth, and $+1.6^\circ\text{C}$ (± 0.2) for T_{soil} at 50-cm depth. Humidity was regulated to achieve equivalent RH between treatments; as such, the absolute humidity (e.g. dew point) was higher in the warmed relative to the ambient treatment, but the vapor pressure deficit (VPD) was also higher in the warmed relative to the ambient treatment. This was done purposefully to simulate climate predictions (Soden & Held, 2006).

We also implemented an extreme experimental heatwave during the early Austral summer (November 2016), where six chambers were exposed to a daily maximum T_{air} of $43\text{--}44^\circ\text{C}$ for 4 consecutive days (Drake *et al.*, 2018). During this time, six chambers were exposed to a reference condition with a daily maximum T_{air} of 28°C . The experiment was terminated on 18 November 2016, when the rapidly-growing trees had grown to c. 9 m in height and 6 cm diameter (Drake *et al.*, 2018). Given the warming treatment, the experimental heatwave, and natural variation, this experiment generated considerable variation in T_{air} . Values for 30-min averages of T_{air} ranged from -1.1 to 45.0°C , while daily-average daytime T_{air} values ranged from 3.4 to 34.5°C . This provided an opportunity to evaluate the potential for thermoregulation of T_{leaf} across a wide range of environmental conditions.

All trees were irrigated equally every 2 wk with Sydney tap water at half of the mean monthly rainfall for this location, apart from the month before the heatwave experiment, when irrigation was suspended (Drake *et al.*, 2018). Rainfall in this location is approximately evenly distributed throughout the year, with a

mean annual value of c. 900 mm. The watering regime resulted in adequate plant-available moisture; soil volumetric water content was equivalent between treatments and well above the wilting point of the soil (Supporting Information Fig. S1). Pre-dawn leaf water potentials were measured with a Scholander-type pressure chamber (1505D-EXP; PMS Instrument Co., Albany, OR, USA) on three leaves per tree on three dates, resulting in a mean value of -0.2 MPa that did not differ between treatments (Table 1; $P > 0.1$). Stomatal conductance (g_s) was measured on three leaves per tree during the mid-morning on 12 dates using three cross-calibrated gas analyzers (LI-6400XT; Li-Cor Inc., Lincoln, NE, USA) under *in-situ* environmental conditions and high light ($1800 \mu\text{mol m}^{-2} \text{s}^{-1}$; g_s was not correlated with soil water content and was an average of $0.34 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ at VPD values from 0.8 to 1.2 kPa (Table 1)). These data suggest that the trees were not water stressed during this experiment.

Canopy temperature measurements

We measured T_{leaf} with continuous infrared measurements of the upper canopy ($T_{\text{L-IR}}$) and automated thermocouple measurements ($T_{\text{L-TC}}$). An infrared radiometer (SI-111; Apogee Instruments, Logan, UT, USA; emissivity set to 0.97) was mounted inside the sun-facing northern side of each whole-tree chamber and pointed at an area of dense foliage in the upper third of the canopy for each tree; the sensors were moved upwards as the trees grew taller. These sensors integrate over an area of c. 1 m^2 and thus measure a temperature averaged across many leaves. We refer to these infrared data as $T_{\text{L-IR}}$. Fine-wire thermocouples (type T, 0.13 mm diameter, 36 AWG, Model 5SRT; Omega Engineering, Norwalk, CT, USA) were installed on the abaxial surface of two upper canopy leaves per tree. We made very small holes through the abaxial epidermis with fine needles and installed the thermocouples such that the junction was inside the leaf and touching the mesophyll. We secured the thermocouple wire to the petiole with twist ties. This installation was delicate but avoided artifactual effects of tape or glue on measured T_{leaf} . We investigated and could not find an effect of thermocouple installation on T_{leaf} as measured by a thermal camera (Model T640; FLIR Systems, Wilsonville, OR, USA). We refer to these thermocouple measurements as $T_{\text{L-TC}}$. These measurements required daily attention to ensure that the thermocouples maintained physical contact with the leaf mesophyll, so it was impractical to measure $T_{\text{L-TC}}$ throughout the 265-d experiment. We

Table 1 Stomatal conductance (g_s) and pre-dawn leaf water potential values for *Eucalyptus parramattensis* trees; values reflect the mean ($\pm 1\text{SE}$) of six trees per treatment.

Temperature treatment	$g_s (\text{mol H}_2\text{O m}^{-2} \text{s}^{-1})^{\text{a}}$	Pre-dawn leaf water potential (MPa)
Ambient	0.31 (0.06)	-0.27 (0.03)
Warmed (+3°C)	0.37 (0.04)	-0.20 (0.04)

^a*In-situ* measurements during mesic atmospheric conditions, where the vapor-pressure deficit of the leaf was between 0.8 and 1.2 kPa.

employed the $T_{\text{L-TC}}$ method during two separate week-long $T_{\text{L-IR}}$ methodology as a continuous measurement of T_{leaf} . To examine the effect of incident light as a driver of T_{leaf} , we also instrumented a subset of two leaves on two trees with c. 5-mm wide gallium arsenide photodiode light sensors mounted on each leaf parallel to the leaf lamina and c. 1 cm away from the leaf thermocouple (Fig. S2).

To address whether the infrared method provided a reliable measurement of actual T_{leaf} (rather than branch or infrastructure temperatures) we measured $T_{\text{L-IR}}$ and $T_{\text{L-TC}}$ on the same group of leaves in all trees during two week-long test campaigns (during June and September 2016). For these tests and in all trees, two leaves in the patch measured by the infrared radiometer were also instrumented with thermocouples. This provided simultaneous $T_{\text{L-IR}}$ and $T_{\text{L-TC}}$ measurements of a common group of leaves. $T_{\text{L-TC}}$ and $T_{\text{L-IR}}$ were both strongly correlated with T_{air} (Fig. S3a,b; $r^2 > 0.9$), and $T_{\text{L-TC}}$ and $T_{\text{L-IR}}$ were strongly positively correlated with each other with a slope approaching unity (Fig. S3c). These results suggest that the continuous infrared measurements ($T_{\text{L-IR}}$) throughout the 265-d experiment provide an accurate measurement of T_{leaf} , rather than branch or infrastructure temperature, so we use this $T_{\text{L-IR}}$ dataset to calculate assimilation-weighted leaf temperatures for comparison with the oxygen isotopic methods (see below, ‘Wood cellulose $\delta^{18}\text{O}$ composition’ section).

CO_2 flux measurements

The net exchange of CO_2 between each tree crown and its surrounding atmosphere was measured every 15 min using a mass balance approach (Barton *et al.*, 2010). The data are similar in nature and quality to previous experiments utilizing this facility (Duursma *et al.*, 2011, 2014; Barton *et al.*, 2012; Drake *et al.*, 2016, 2018). Net CO_2 flux measurements began on 28 February 2016 when a suspended plastic floor was installed at c. 65-cm height above the soil surface and sealed around the stem of each tree, which isolated the crown flux measurements from CO_2 and H_2O efflux from the soil. We recorded 265 d of net CO_2 flux measurements for all 12 trees as they grew rapidly from an average height of 230 to 850 cm.

We calculated the assimilation-weighted leaf temperature ($T_{\text{L-AW}}$) for each chamber on each day, utilizing the continuous measurements of canopy temperature ($T_{\text{L-IR}}$) and net CO_2 flux. The $T_{\text{L-AW}}$ is a metric of mean T_{leaf} weighted by net CO_2 flux, and thus represents the temperature at which most of the photosynthesis occurred. We utilized the ‘weighted.mean’ function within the STATS R package to calculate $T_{\text{L-AW}}$. Following (Helliker & Richter, 2008), nighttime data were excluded from this calculation; only data associated with a light intensity (photosynthetic photon flux density; PPFD) exceeding $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ were used.

Wood cellulose $\delta^{18}\text{O}$ composition

All trees were felled at the end of the experiment (18 November 2016) and a 1-cm thick transverse section of the stem was collected from each tree at 65-cm height. We identified three

periods of time, each *c.* 35 d in duration, with strongly contrasting average T_{air} values (June, August and November 2016). We used a weekly dataset of directly-measured stem diameters at this position on the stem of each tree (65-cm height) to identify the radial position on each stem section that corresponded to wood that was produced during these three periods of time. Wood samples from these locations were removed with a motorized drill, and α -cellulose was extracted following the Brendel procedure modified with an addition of 17% NAOH wash to remove hemicellulose (Brendel *et al.*, 2000; Gaudinski *et al.*, 2005) as described in Song *et al.* (2014). Extracted α -cellulose was analyzed for $\delta^{18}\text{O}$ composition at The Centre for Stable Isotope Biogeochemistry, University of California, Berkeley.

$T_{\text{L-AW}}$ was estimated from cellulose $\delta^{18}\text{O}$ composition using a rearrangement of the Craig–Gordon model (Craig & Gordon, 1965) to calculate the vapor pressure inside the leaf (e_i ; Helliker & Richter, 2008; Song *et al.*, 2011):

$$e_i = \frac{(\Delta_V - \varepsilon_k)e_a}{\left(\frac{(\Delta_{\text{cel}} - \varepsilon_C)\wp}{(1 - p_{\text{ex}}p_x)(1 - e^{-\wp})} \right) - \varepsilon^+ - \varepsilon_k} \quad \text{Eqn 1}$$

where Δ_V is the $\delta^{18}\text{O}$ of atmospheric water vapor relative to source water, ε_k is the kinetic fractionation for combined diffusion through the stomata and the boundary layer (assumed to be 28‰), e_a is the ambient vapor pressure (measured directly by the WTCs), Δ_{cel} is the $\delta^{18}\text{O}$ of cellulose relative to source water, ε_c is the equilibrium fractionation factor between organically bound oxygen and synthesis water (assumed to be 27‰), \wp is the Péclet number, p_{ex} is the number of organically bound oxygen atoms in leaf-formed sucrose that exchange with xylem water during cellulose synthesis, p_x is the proportional deviation of the isotope ratio of xylem water from source water, and ε^+ is the equilibrium fractionation between liquid water and vapor, calculated as in Cernusak *et al.* (2016). We assume that the product of p_{ex} and p_x is 0.4 (Song *et al.*, 2011, 2014) and that is constant at 0.08 (Helliker & Richter, 2008).

The isotopic composition of branch water, assumed to be equal to source water, was measured for two small branches (*c.* 2-cm diameter) sampled from each tree at harvest. Water was cryogenically extracted (Loucos *et al.*, 2015) and $\delta^{18}\text{O}$ measured after vaporization using a water vapor isotope analyzer (TIWA-45EP; Los Gatos Inc., Mount View, CA, USA) coupled to a vaporizer (Water Vapor Isotope Standard Source; Los Gatos Inc.) and a dry air source (Dry Air Source; Los Gatos Inc.). The analyser was calibrated using three secondary standards with $\delta^{18}\text{O}$ values of −14.4, −1.5 and 34.1‰.

We assessed the need to correct for interference by volatile organic compounds on the laser absorption measurements (as described by West *et al.*, 2010) through analysis of 13 samples (seven branch samples and six leaf water samples collected at harvest) on an isotope ratio mass spectrometer at The University of California, Berkeley. The relationship between laser absorption measurements and the isotope ratio mass spectrometer was established from standard least squares regression and product–moment correlation. The slope of the line was tested for significant

difference from 1 using the Student's *t*-test, where $t = (1 - \text{slope})/\text{standard error of the slope}$ (Barbour *et al.*, 2001). There was no significant difference between the slope and 1 ($t = 1.4$, $P = 0.2$). There was a significant difference between the intercept and zero ($t = -2.3$, $P = 0.04$), resulting in extracted branch water samples analyzed by laser absorption being positively offset by +1.12‰ ($y = 0.93x + 1.12$, $r^2 = 0.98$, data not shown). For $\delta^{18}\text{O}$ of extracted branch water, there were no statistically significant differences between trees, so an average value was used as the $\delta^{18}\text{O}$ of source water (−3.28‰) for all trees. This value aligns with long term $\delta^{18}\text{O}$ precipitation measurements of the Sydney Basin (Hughes & Crawford, 2013), which provides the water used to irrigate the trees.

The stable isotope composition of water vapour within each chamber was monitored over 82 d from late August to mid-November using the TIWA-45EP plumbed into the chamber system. A valve system (Multiport Inlet Unit; Los Gatos Inc.) was used to sequentially sample each chamber for 4.5 min every h. The laser was calibrated once per week using the International Atomic Energy Agency (IAEA) standards Vienna Standard Mean Ocean Water (VSMOW), Standard Light Antarctic Precipitation (SLAP) and Greenland Ice Sheet Precipitation (GISP). Over the November period, within-chamber vapour $\delta^{18}\text{O}$ during daylight hours was −12.8‰ in the ambient chambers and −11.8‰ in the warmed chambers on average. These values were used in Eqn 1 for all wood sampling dates.

Finally, $T_{\text{L-AW}}$ was estimated from e_i , assuming that the leaf airspaces were fully saturated with water vapor (Helliker & Richter, 2008):

$$T_{\text{L-AW}} = \frac{240.97 \left(\log \frac{e_i}{0.61365} \right)}{17.502 - \left(\log \frac{e_i}{0.61365} \right)} \quad \text{Eqn 2}$$

Leaf energy balance modeling

We used a standard leaf-scale photosynthetic model to predict how the leaf to air temperature difference ($T_{\text{leaf}} - T_{\text{air}}$) would vary in response to radiation absorption and latent cooling driven by VPD and PPF. We used the model described in a study by Duursma (2015), which couples a standard model of photosynthetic biochemistry (Farquhar *et al.*, 1980) with a model of stomatal conductance (Medlyn *et al.*, 2011) and leaf energy balance (Wang & Leuning, 1998). Specifically, we utilized the 'PhotosynEB' function within the PLANTECOPHYs R package (Duursma, 2015) to predict T_{leaf} under combinations of PPF (0 to 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and VPD (0 to 2 kPa) with a leaf width of 3 cm, a moderate wind speed of 0.5 m s^{-1} , and T_{air} of 20°C. We used a stomatal conductance parameter (g_1) of 4, based on direct leaf-scale measurements in this experiment as well as previous work from a similar *Eucalyptus* species at this location (Gimeno *et al.*, 2016). We separately tested the impact of wind speed in model simulations that used the measured values for

PPFD, VPD, and T_{air} , with wind speed values of 0.1, 0.5, 1 and 5 m s⁻¹. The Duursma (2015) model predicts $T_{\text{leaf}} - T_{\text{air}}$ using a standard approach:

$$T_{\text{leaf}} - T_{\text{air}} = \frac{R\gamma \left[\frac{g_{b,h}}{g_w} \right]}{\rho_a c_p g_{b,h} \left[s + \gamma \left[\frac{g_{b,h}}{g_w} \right] \right]} - \frac{\text{VPD}}{s + \gamma \left[\frac{g_{b,h}}{g_w} \right]} \quad \text{Eqn 3}$$

where R is net radiation (W m⁻²), γ is the psychrometric constant, $g_{b,h}$ is the boundary layer conductance to heat (m s⁻¹), g_w is the conductance for water vapor (m s⁻¹), ρ_a is the density of air (kg m⁻³), c_p is the specific heat capacity of air (J kg⁻¹ K⁻¹), s is the slope of the saturated vapor pressure curve relative to temperature (Pa K⁻¹), and VPD is the vapor pressure deficit (kPa). Note that leaf physiological processes of photosynthesis and stomatal conductance are simulated simultaneously and influence $T_{\text{leaf}} - T_{\text{air}}$ via g_w .

Statistical analyses

The inferences presented here are primarily based on the relationships of T_{leaf} vs T_{air} and $T_{\text{L-AW}}$ vs T_{air} . We used analysis of covariance (ANCOVA) to test for differences in slope and intercept between the ambient and warmed treatments, recognizing that we only have 12 unique experimental units. As such, we utilized mixed-effects ANCOVAs with random slope and intercept terms for each WTC ($n = 12$). This allowed for robust calculations of the overall slopes without the pseudoreplication that would occur if we assumed each observation to be independent. We fit mixed effects ANCOVAs with the LME4 R package, utilizing fixed effects of T_{air} and warming treatment with random slopes and intercepts for each tree, using data from the entire 265 d period, including the heatwave components. Model selection based on AIC values and log-likelihood was used to assess whether the ambient and warmed treatments had equivalent slope and intercept terms. We calculated the 95% confidence intervals of the slope estimates to infer the degree of thermoregulation (Fig. 1) utilizing the ‘confint’ R function.

Results

Leaf temperature variation

$T_{\text{L-IR}}$ varied between -0.8 and 45.7°C in a manner that was strongly and linearly correlated with T_{air} across > 300,000 direct measurements at 15-min resolution (Fig. 2a; $y = 0.03 + 1.026x$, $r^2 = 0.98$, $P < 0.001$). The slope of this line was slightly above unity (95% confidence interval from 1.01 to 1.03). The leaf to air temperature difference ($T_{\text{L-IR}} - T_{\text{air}}$) ranged from -2 to 9°C but was most frequently in the -1 to 3°C range (Fig. 2b). In fact, $T_{\text{L-IR}}$ was within 1°C of T_{air} 60% of the time during the day and 97% of the time during the night (80% across all observations). Variation in $T_{\text{L-IR}} - T_{\text{air}}$ corresponded to the environmental drivers of PPFD and VPD, reflecting the balance between radiative heating and evaporative cooling (Fig. 2c). $T_{\text{L-IR}}$ exceeded

T_{air} most strongly under conditions of high radiative input but low evaporative cooling (high PPFD and low VPD), while $T_{\text{L-IR}}$ was < T_{air} during conditions of low radiative input but high evaporative cooling (low PPFD and high VPD, notably at night). These observed responses of $T_{\text{L-IR}} - T_{\text{air}}$ to PPFD and VPD were consistent with the predictions of a standard leaf energy balance model (Fig. S4).

Fine-scale measurements of $T_{\text{L-TC}}$ and PPFD incident on individual leaves provided further evidence that $T_{\text{L-IR}} - T_{\text{air}}$ was driven by light intensity and the shading of surrounding leaves (Fig. 3a). Short-term (1-min resolution) variation in PPFD incident on individual leaves was strongly associated with T_{leaf} variation of c. 3°C, with little variation in T_{air} (Fig. 3a). Across the two week-long test periods, $T_{\text{L-IR}} - T_{\text{air}}$ was positively correlated with top-canopy PPFD (Fig. 3b; $y = 0.207 + 0.0025x$, $P < 0.001$, $r^2 = 0.51$). There was substantial leaf-to-leaf variation in temperature (Fig. 3b), presumably as a result of the complexities of light transmission through tree canopies and leaf-to-leaf variation in boundary layer conductance. Under conditions of high photosynthetic light availability, T_{leaf} was often c. 1–3°C higher than T_{air} , although individual leaves were as much as 9°C higher than T_{air} in some rare cases (Fig. 3b).

Net CO₂ fluxes and assimilation-weighted T_{leaf}

The whole-tree chambers provided a direct and continuous measurement of net CO₂ fluxes for entire tree crowns (Fig. 4). Net CO₂ flux was negative at night, reflecting net CO₂ emission from trees to the atmosphere via autotrophic respiration. Net CO₂ flux during the day was generally positive and responsive to environmental drivers, reflecting net photosynthetic CO₂ uptake.

The diurnal time-course of net CO₂ uptake influenced $T_{\text{L-AW}}$ relative to T_{air} . $T_{\text{L-IR}}$ closely followed T_{air} at this diurnal scale (Fig. 4a,c,e), as expected from the strong and nearly 1 : 1 relationship between $T_{\text{L-IR}}$ and T_{air} throughout the experiment (Fig. 2a). On relatively cold days (Fig. 4a,b), net CO₂ uptake was maximal during the warmest periods of the day, which also had high PPFD and moderate VPD such that T_{leaf} tended to exceed T_{air} and $T_{\text{L-IR}}$ by 1–3°C. As such, $T_{\text{L-AW}}$ exceeded daily average T_{air} and $T_{\text{L-IR}}$. On this example cool day (Fig. 4a,b), daily average T_{air} was 16.9°C, while $T_{\text{L-AW}}$ was 18.5°C and $T_{\text{L-IR}}$ was 17.87°C. On days with moderate temperatures (Fig. 4c,d), net CO₂ uptake was maximal during the late morning, when PPFD levels were high but T_{air} values had not yet reached their maximum. On such days, $T_{\text{L-AW}}$ and daytime average T_{air} and $T_{\text{L-IR}}$ tended to be similar. On this example moderate day (Fig. 4c,d), daily average T_{air} was 25.8°C, while $T_{\text{L-AW}}$ was 26.1°C and $T_{\text{L-IR}}$ was 26.0°C. On hot days, canopy CO₂ uptake was maximal during the morning only (Fig. 4e,f). CO₂ fluxes dropped to nearly zero by the late morning and remained low throughout the afternoon. On some days there was a brief period of CO₂ uptake in the evening, when T_{air} declined but PPFD was still available (Fig. 4e,f). On these hot days, $T_{\text{L-AW}}$ was lower than daytime average T_{air} and $T_{\text{L-IR}}$. On this example hot day, (Fig. 4e,f), daily average T_{air} was 30.6°C, while $T_{\text{L-AW}}$ was 26.2°C and $T_{\text{L-IR}}$ was 30.8°C.

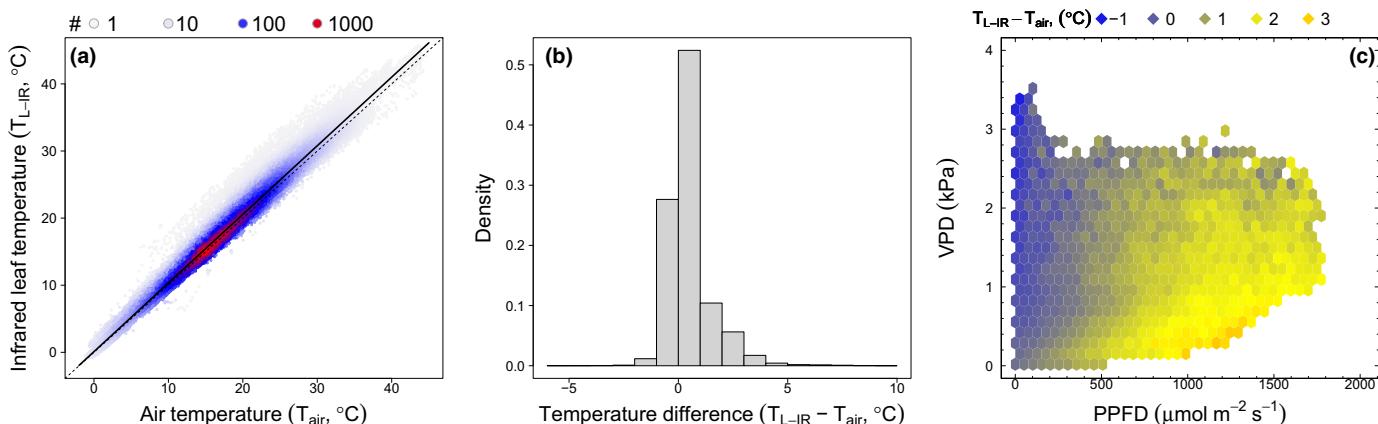


Fig. 2 The correspondence between leaf temperature as measured by the infrared radiometers (T_{L-IR}) and air temperature (T_{air}) for *Eucalyptus parramattensis*. More than 300 000 data points measured at 15-min resolution are summarized here, including daytime and nighttime data. T_{L-IR} was strongly positively correlated with T_{air} (a); the black dashed line reflects the 1 : 1 relationship, and the solid black line reflects the best fit to the data ($y = 0.06 + 1.03x$, $r^2 = 0.98$, $P < 0.001$). Panel (a) visualizes the data with a density plot; grey symbols reflect a low density of points, blue symbols reflect a moderate density of points, and red symbols reflect the highest density of points. The leaf to air temperature difference ranged from c. -5 to 10 °C but was most frequently in the -1 to 3 °C range (b). Variation in the leaf to air temperature difference was related to the environmental drivers of photosynthetic photon flux density (PPFD) incident at the top of the chambers and vapor pressure deficit (VPD); (c). For (c), hexagons containing at least twenty observations are shown, with the color reflecting the mean $T_{L-IR} - T_{air}$.

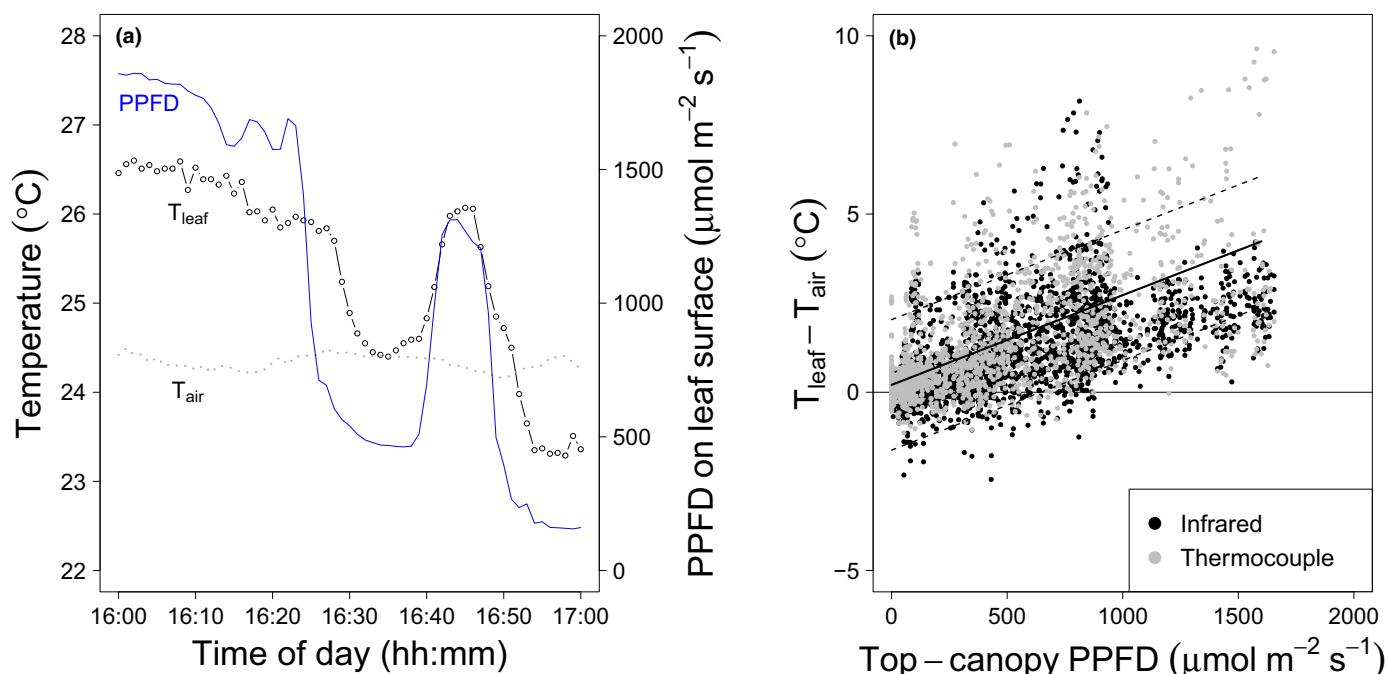


Fig. 3 Illustration of the effect of incident light on leaf temperature (T_{leaf}) for *Eucalyptus parramattensis*. During a short test (1 h) experiment (a), the chamber air temperature (T_{air}) was set at 24.5 °C and T_{leaf} was measured each minute along with the photosynthetic photon flux density (PPFD) incident on the surface of a single leaf. Values reflect minute-by-minute measurements during the afternoon of 27 September 2016. Throughout both week-long tests of all trees ($n = 12$) when leaves were fitted with thermocouples (b), the difference between T_{leaf} and T_{air} increased with PPFD as measured at the top of the canopy. The solid grey line was fit to the thermocouple data, and the dashed grey lines reflect the 95% confidence interval.

We move beyond these three examples and present daytime average T_{leaf} , T_{air} , and T_{L-AW} for all 265 d of the measurement period. Daytime average T_{L-IR} and T_{air} were strongly positively correlated (Fig. 5a; overall fit: $y = 1.21 + 0.98x$, $P < 0.001$, $r^2 = 0.97$). There was some evidence that the warmed treatment had a lower slope than the ambient

treatment (mixed model ANCOVA, $P = 0.02$), but a simple model with common slope and intercept terms across treatments was preferred on the basis of AIC scores (6350 for common slope and intercept, 6532 for separate slopes and intercepts). The 95% confidence intervals for the slope included 1.0 for both treatments when analyzed separately

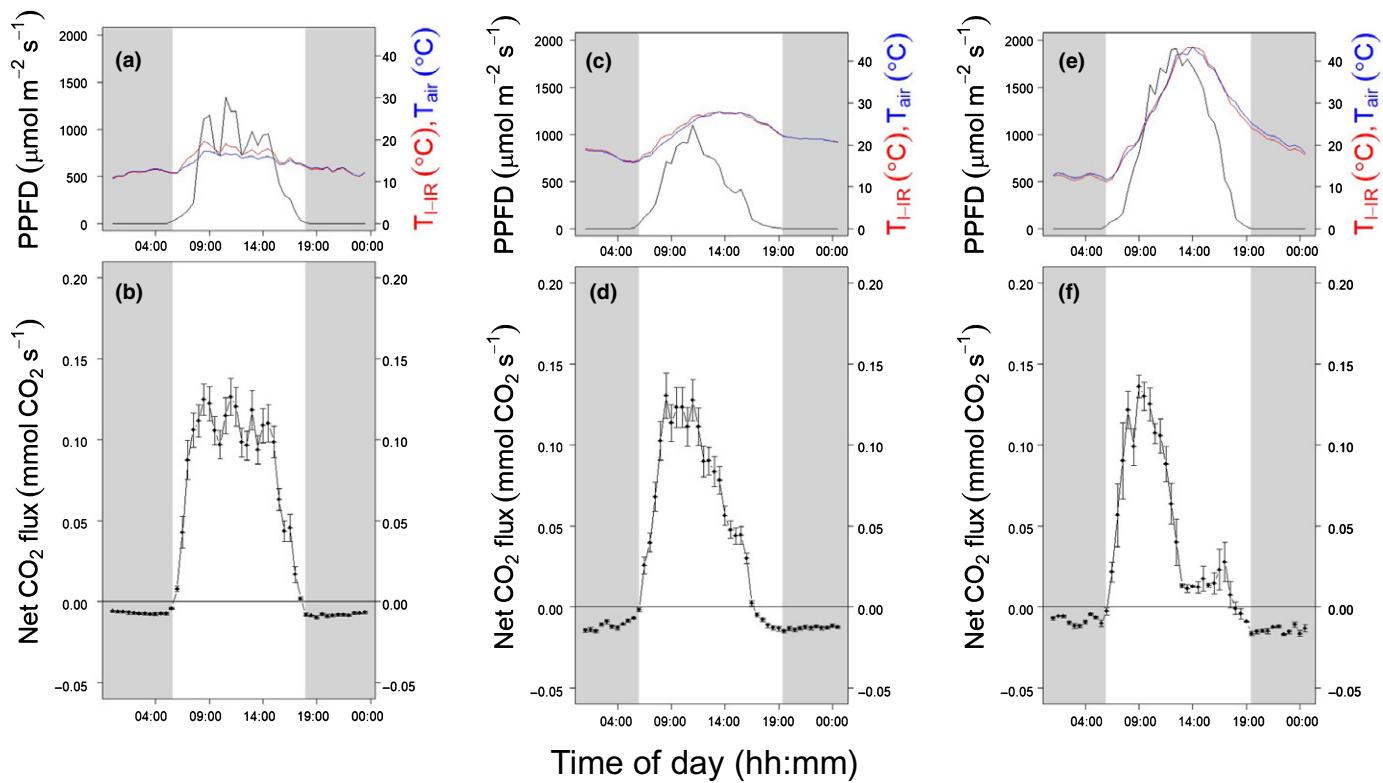


Fig. 4 Example diurnal time courses of incident top-canopy photosynthetic photon flux density (PPFD), infrared measurements of canopy temperature (T_{L-IR}), air temperature (T_{air}), and net CO_2 fluxes for whole-tree crowns of *Eucalyptus parramattensis*. We show an example low temperature day (a, b), where the average daytime air temperature (T_{air}) was 16.9°C , an example moderate temperature day (c, d), where the average daytime T_{air} was 25.8°C , and an example extremely hot temperature day (e, f) where the average daytime T_{air} was 30.6°C . For illustrative purposes, we show the mean (\pm SE) of the ambient temperature treatment only ($n = 6$).

(95% CI from 0.98 to 1.05 in ambient, 0.91 to 1.01 for warmed, 0.96 to 1.02 for the simple model combining treatments). These results suggest that T_{leaf} was primarily driven by T_{air} at this daily scale, with no evidence of thermoregulation to maintain homeostatic T_{leaf} values. The intercept term was significantly positive (95% CI from 0.47 to 1.9°C); combined with a slope indistinguishable from unity, this indicates that leaves tended to be slightly warmer than the air.

T_{L-AW} was also strongly positively correlated with T_{air} across all 265 d (Fig. 5b; overall fit: $y = 4.24 + 0.89x$, $P < 0.001$, $r^2 = 0.92$). There was marginal evidence of a lower slope in the warmed relative to the ambient treatment (mixed effects ANCOVA, $P = 0.05$), but again a simple model with a single slope and intercept term was preferred on AIC scores (9839 for common slope and intercept, 9844 for separate slopes and intercepts). The slope of this relationship was significantly lower than 1.0 in both treatments when analyzed separately (95% CI from 0.88 to 0.98 in the ambient treatment, 0.78 to 0.92 in warmed treatment, 0.84 to 0.93 for the preferred simple model combining treatments). This indicates a degree of uncoupling between T_{air} and T_{L-AW} , driven by the different diurnal time-courses of T_{air} and PPFD, and the resulting CO_2 assimilation (Fig. 4). Averaged across the entire experiment, assimilation-weighted T_{leaf} averaged 20.9°C in the ambient treatment and 23.5°C in the warmed treatment.

The calculations of T_{L-AW} via the cellulose $\delta^{18}\text{O}$ approach were strongly correlated with direct measurements of T_{leaf} . The wood cellulose $\delta^{18}\text{O}$ composition ranged from 29.6 to $34.9\text{\textperthousand}$, with an average of $33.27\text{\textperthousand}$. T_{L-AW} inferred from cellulose $\delta^{18}\text{O}$ sampled over three time periods were strongly correlated with direct daytime measurements of T_{L-IR} , although there was some positive bias, particularly at low temperatures (Fig. 6a). The 95% confidence interval of this slope was substantially < 1.0 (0.62 to 0.85). This is consistent with the differential ‘sampling’ of leaf temperatures by photosynthesis (Figs 4, 5b). T_{L-AW} values inferred from cellulose $\delta^{18}\text{O}$ were also strongly correlated with T_{L-AW} values as calculated by the CO_2 flux measurements in a manner that approached the 1 : 1 line (Fig. 6b). The slope was 0.9 but the 95% confidence interval of this slope included 1.0 (0.86 to 1.12). Thus, the cellulose $\delta^{18}\text{O}$ calculations and the direct measurements of T_{leaf} and T_{L-AW} indicate that leaf temperatures strongly varied over time, driven primarily by variation in T_{air} .

Discussion

We combined continuous measurements of T_{leaf} and the net CO_2 exchange of whole-tree crowns to calculate assimilation-weighted T_{leaf} (T_{L-AW}) and compared these measurements to T_{L-AW} calculated from cellulose $\delta^{18}\text{O}$. The strong correspondence

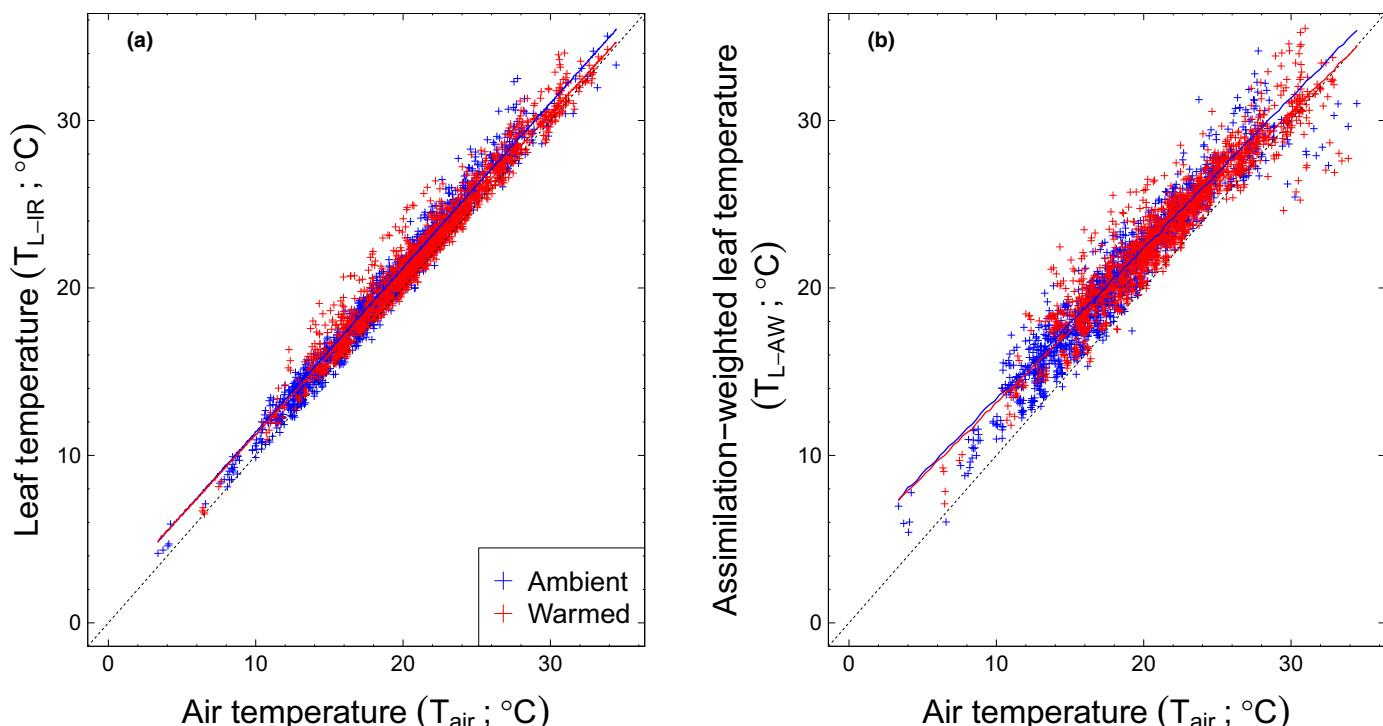


Fig. 5 Daily daytime averages for leaf temperatures measured by infrared radiometers ($T_{L\text{-IR}}$), air temperatures (T_{air}), and assimilation-weighted T_{leaf} ($T_{L\text{-AW}}$) calculated from CO_2 fluxes and $T_{L\text{-IR}}$ for *Eucalyptus parramattensis*. Data points reflect daily data for individual chambers ($n = 12$) across 265 d of measurements. The black dashed line reflects the 1 : 1. Solid colored lines reflect the fitted estimate of a mixed effects ANCOVA and dashed lines reflect the 95% CI. Daily average T_{leaf} was strongly and linearly correlated with daily average T_{air} with a slope equivalent to unity (slope of 0.98; a). Assimilation-weighted T_{leaf} ($T_{L\text{-AW}}$) was also strongly correlated with T_{air} (b), but with a slope that was significantly less than unity (slope of 0.89).

between these independent estimates of $T_{L\text{-AW}}$ (Fig. 6b) illustrates the utility of oxygen isotopes for an integrated assessment of the thermal biology of trees. In this experiment, the thermal biology of these *E. parramattensis* trees was highly dynamic and variable, with T_{leaf} responding to T_{air} , PPF and VPD across timescales of minutes to seasons. Critically, the dynamic nature of T_{leaf} was imprinted on the cellulose $\delta^{18}\text{O}$ record, but not directly – there was a differential ‘sampling’ of T_{leaf} driven by the temperature dependence of photosynthesis. We found direct evidence of moderation of $T_{L\text{-AW}}$ relative to T_{air} (Fig. 6a), a core concept of Helliker & Richter (2008), but this moderation was modest and did not lead to a constant $T_{L\text{-AW}}$. We conclude that *E. parramattensis* is best characterized as nearly poikilothermic – as it exhibits little thermoregulation of T_{leaf} – and that the cellulose $\delta^{18}\text{O}$ methodology can provide an excellent integrated assessment of photosynthetically-weighted T_{leaf} .

This work is consistent with a large body of literature regarding the biophysical controls of leaf temperature. We recognize that many aspects of plant morphology and physiology can influence T_{leaf} relative to T_{air} , including leaf width, foliar clumping, canopy-scale influences on atmospheric mixing, and latent cooling from transpiration (Jarvis & McNaughton, 1986; Hadley & Smith, 1987; Smith & Carter, 1988; Leuzinger & Körner, 2007; Leigh *et al.*, 2012; Rundel *et al.*, 2017; Wright *et al.*, 2017; Drake *et al.*, 2018). However, the evidence presented here emphasizes the strong environmental control of T_{air} on T_{leaf} . We do not argue that T_{leaf} is always equal to T_{air} , but that variation in T_{leaf} is strongly driven by the environment – including T_{air} – and can be

predicted from well-known biophysical mechanisms (Gates, 1965; Campbell & Norman, 2000; Jones, 2013). Furthermore, large deviations of T_{leaf} relative to T_{air} were rare in this experiment. Thus, we suggest that our study trees are best considered as nearly poikilothermic, rather than ‘limited homeotherms’. The lack of strong thermoregulation of T_{leaf} observed here emphasizes the importance of physiological and metabolic acclimation as mechanisms for plants to cope with a variable thermal environment.

There were some patterns of deviation between T_{leaf} and T_{air} that likely reflect a degree of biologically meaningful temperature regulation. During the extreme heatwave conditions, trees transpired more than expected, such that T_{leaf} values were an average of 2.8 °C cooler than expected (Drake *et al.*, 2018). This latent cooling of T_{leaf} contributed to the avoidance of thermal damage in these trees. Thus while we emphasize the commonality between T_{leaf} and T_{air} observed across the vast majority of this experiment, we recognize that heterothermy may be important in some situations (Drake *et al.*, 2018; Blonder & Michaletz, 2018).

This study had several limitations that made it an imperfect test of work by Helliker & Richter (2008), a landscape-scale analysis of tree ring cellulose from sites across North America. This study would have more rigorously tested the findings of Helliker & Richter (2008) if it were repeated across a range of sites, particularly boreal forests where Helliker & Richter (2008) predicted the strongest divergence between $T_{L\text{-AW}}$ and T_{air} . The average $T_{L\text{-AW}}$ values reported here (20.9 and 23.5 °C in the ambient and warmed treatments, respectively) are within the reported range

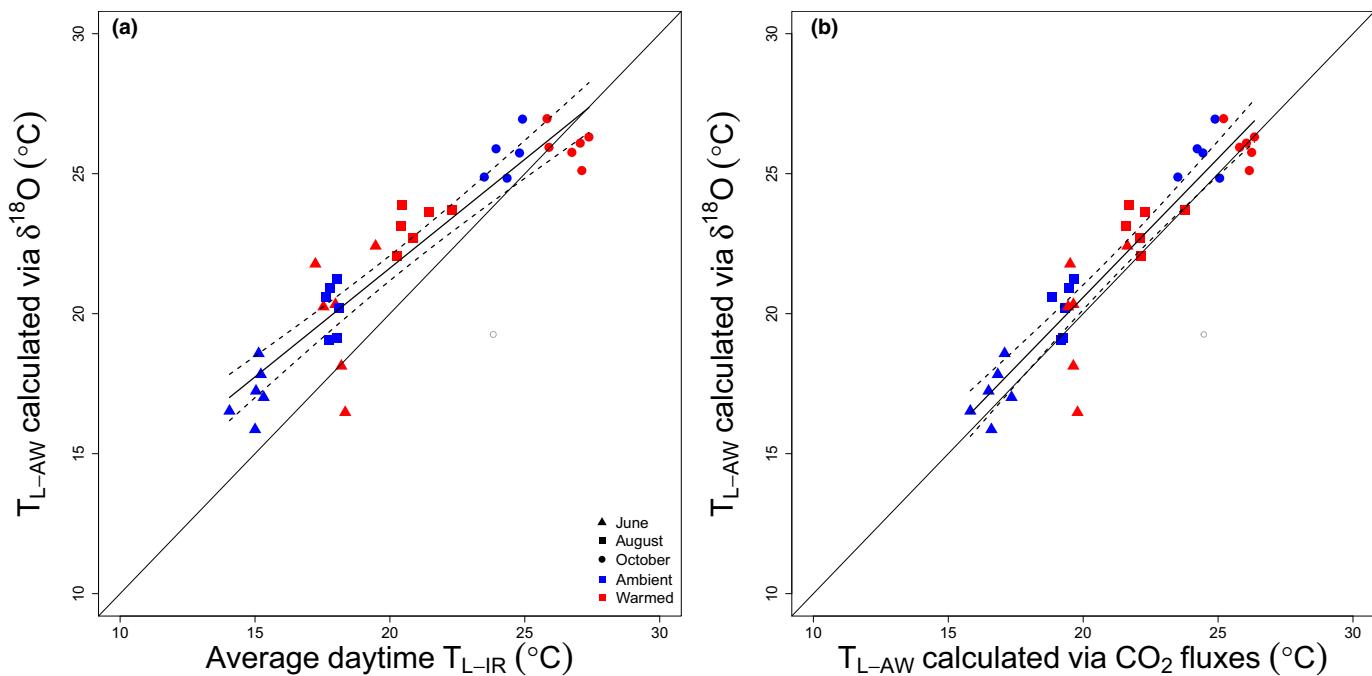


Fig. 6 Agreement between direct measurements of leaf temperature and inference from oxygen isotopes for *Eucalyptus parramattensis*. Data points reflect individual chambers ($n = 12$) sampled in three seasons, and the solid grey line reflects the 1 : 1 relationship. Solid black lines reflect the fitted estimate of a simple linear model and dashed lines reflect the 95% CI. Assimilation-weighted leaf temperature via the cellulose $\delta^{18}\text{O}$ approach ($T_{\text{L-AW}}$) was linearly correlated with direct measurements of T_{leaf} ($T_{\text{L-IR}}$; a), but with a slope lower than unity ($y = 6.08 + 0.77x$, $r^2 = 0.86$, $P < 0.001$). $T_{\text{L-AW}}$ via cellulose $\delta^{18}\text{O}$ was linearly correlated with $T_{\text{L-AW}}$ via the CO₂ flux approach (averaged for the same 35-d period as the cellulose method) with less bias (b; $y = 0.73 + 0.99x$, $r^2 = 0.88$, $P < 0.001$). The 95% confidence interval of the slope in (b) included 1.0 (0.86 to 1.12). The open grey circle reflects an outlier that was not included in the linear regressions.

across boreal and sub-tropical regions based on cellulose $\delta^{18}\text{O}$ ($21.4 \pm 2.2^\circ\text{C}$; Helliker & Richter, 2008; Song *et al.*, 2011). Thus, the average data across 265 d reported here are consistent with Helliker & Richter (2008); however, the warming treatment effect on long-term integrated $T_{\text{L-AW}}$ as well as the data reported here on shorter time-scales are not consistent with the concept of homeostatic T_{leaf} . This suggests an important component of temporal scale in the interpretation of these datasets.

A second limitation to a rigorous test of the Helliker and Richter approach is that the within-chamber airspaces were continuously mixed by air-handling units in this study (average air speeds of $c. 0.3 \text{ m s}^{-1}$), which was required for the measurement of net CO₂ fluxes and for the control of T_{air} and CO₂ concentration (Barton *et al.*, 2010). Thus, it is possible that T_{leaf} was more strongly coupled to T_{air} in these chambers than it would have been outside the chambers. Our energy balance simulations across a range of wind speeds indicate that T_{leaf} was predicted to be higher under low wind speed, but T_{leaf} still varied substantially and nearly linearly with T_{air} (Fig. S5). Thus, we suggest that an altered boundary layer conductance from the air-handling circulation in this study is unlikely to have altered the lack of thermoregulation observed here.

While recognizing these limitations, we suggest that our study has made useful progress towards combining the biophysical and isotopic literature regarding leaf temperature, and clarified how T_{leaf} varies in relation to T_{air} at different levels of scale and integration. We particularly note the strong correlation between our

measurements of $T_{\text{L-AW}}$ via CO₂ fluxes and cellulose $\delta^{18}\text{O}$. We found remarkable convergence in the estimates of T_{leaf} across temporal variation in T_{air} between the isotopic and direct measurement approaches. Both sets of data indicate that T_{leaf} varies seasonally and in response to warming treatment. The agreement across methods lends further evidence to support the approach of estimating a temporally-integrated assimilation-weighted T_{leaf} from cellulose $\delta^{18}\text{O}$ measurements. We suggest that similar work is warranted in boreal and cold-temperate systems to directly test the prediction that assimilation-weighted T_{leaf} strongly exceeds T_{air} in these systems (Helliker & Richter, 2008). Further, our observation of a strong correlation between T_{air} and T_{leaf} with some deviation driven by the environment is consistent with other site-level observations of trees *in-situ*, including boreal conifers and subtropical broadleaved trees (Ball *et al.*, 1988; Martin *et al.*, 1999; Kim *et al.*, 2018) as well as tropical trees (Rey-Sánchez *et al.*, 2016; Fauset *et al.*, 2018), but we recognize that differences in leaf and crown structure can influence the degree of coupling between T_{air} and T_{leaf} .

Conclusions

We utilized the unique infrastructure of whole-tree chambers to directly quantify T_{leaf} and assimilation-weighted T_{leaf} across a wide range of environmental conditions, and confirmed the utility of cellulose $\delta^{18}\text{O}$ measurements as temporal integrators of plant thermal biology. Collectively, this dataset characterized the

highly dynamic nature of T_{leaf} which varied across timescales with air temperature, radiation fluxes, and latent cooling in a manner consistent with biophysical theory. Thus, we find no evidence of moderation of T_{leaf} towards a homeostatic value, which does not support the concept of plants as strong thermoregulators, at least in this case study. We advocate for similarly detailed assessments of tree thermal biology across climate gradients to robustly assess the generality of thermoregulation in trees.

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Author contributions

JED co-led the experimental design, contributed to data collection for the chamber flux and T_{leaf} datasets, and led the data analysis and writing. RH and MMB led all of the measurement and interpretation of the $\delta^{18}\text{O}$ data, and contributed to writing. AV contributed to the T_{leaf} data collection as well as the experimental design, data analysis, and writing. PBR contributed to the experimental design and writing. CVMB contributed to the measurements of chamber flux and leaf temperature, and contributed to experimental design, data analysis, and writing. MGT was the senior scientific lead; he co-led the experimental design and made large contributions to analysis, interpretation, and writing.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Soil volumetric water content over time.

Fig. S2 Thermal image of *Eucalyptus parramattensis* leaves.

Fig. S3 Leaf and air temperatures during two wk-long campaigns.

Fig. S4 The leaf to air temperature difference ($T_{\text{leaf}} - T_{\text{air}}$) as predicted by a standard leaf energy balance model.

Fig. S5 The effect of windspeed on leaf temperature (T_{leaf}) as predicted by a standard leaf energy balance model.

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