Convergent acclimation of leaf photosynthesis and respiration to prevailing ambient temperatures under current and warmer climates in *Eucalyptus tereticornis*

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**Summary**

- Understanding physiological acclimation of photosynthesis and respiration is important in elucidating the metabolic performance of trees in a changing climate. Does physiological acclimation to climate warming mirror acclimation to seasonal temperature changes?
- We grew *Eucalyptus tereticornis* trees in the field for 14 months inside 9-m tall whole-tree chambers tracking ambient air temperature (*T*<sub>air</sub>) or ambient *T*<sub>air</sub> + 3°C (i.e. ‘warmed’). We measured light- and CO₂-saturated net photosynthesis (*A*<sub>max</sub>) and night-time dark respiration (*R*) each month at 25°C to quantify acclimation. Tree growth was measured, and leaf nitrogen (N) and total nonstructural carbohydrate (TNC) concentrations were determined to investigate mechanisms of acclimation.
- Warming reduced *A*<sub>max</sub> and *R* measured at 25°C compared to ambient-grown trees. Both traits also declined as mean daily *T*<sub>air</sub> increased, and did so in a similar way across temperature treatments. *A*<sub>max</sub> and *R* (at 25°C) both increased as TNC concentrations increased seasonally; these relationships appeared to arise from source–sink imbalances, suggesting potential substrate regulation of thermal acclimation.
- We found that photosynthesis and respiration each acclimated equivalently to experimental warming and seasonal temperature change of a similar magnitude, reflecting a common, nearly homeostatic constraint on leaf carbon exchange that will be important in governing tree responses to climate warming.

**Introduction**

Global mean temperatures are expected to rise 1–4°C by 2050 (IPCC, 2013). As forest ecosystems dominate the terrestrial carbon (C) cycle (Bonan, 2008; Pan et al., 2011), forest responses to climate warming will influence climate–C cycle feedbacks. Earth system models often predict that warming might reduce forest gross primary productivity while increasing autotrophic respiration (Luo, 2007), a response that would weaken the terrestrial C sink and result in a positive feedback that stimulates further warming (Dufresne et al., 2002; Friedlingstein et al., 2014). However, trees have a broad capacity to acclimate photosynthetic and respiratory physiology in response to changes in temperature (Bolstad et al., 2003; Lee et al., 2005; Bruhn et al., 2007; Ov et al., 2008, 2010; Tjoelker et al., 2008, 2009; Slot & Kitajima, 2015; Reich et al., 2016). Such acclimation may reduce the sensitivity of terrestrial C fluxes to changes in temperature, and dampen the positive feedback between warming and rising atmospheric CO₂ (King et al., 2006; Smith & Dukes, 2013; Lombardozzi et al., 2015).

We use the term ‘acclimation’ to describe a change in the instantaneous temperature response of leaf photosynthesis (*A*) and/or dark respiration (*R*) in response to a long-term change (weeks to years) in growth temperature (Atkin et al., 2005), regardless of whether this change is interpreted as beneficial (Way & Yamori, 2014). Evidence for physiological acclimation of leaf CO₂ exchange to temperature comes largely from two types of studies: *in situ* field studies of trees responding to natural seasonal variation in temperature (e.g. Atkin et al., 2000; Lee et al., 2005; Ow et al., 2008, 2010; Tjoelker et al., 2008, 2009); and experimental warming manipulations, mostly with small trees grown in controlled environments for relatively short periods of time (e.g. Bolstad et al., 2003; Bruhn et al., 2007; Cheesman & Winter, 2013; Drake et al., 2015). Are these responses manifestations of a common underlying mechanism, such that studies of seasonal acclimation can be used to infer responses to climate warming, as shown recently for *R* of temperate and boreal trees (Reich et al., 2016)? Or, does climate warming induce a novel physiological response such that climate warming effects on *A* and *R* cannot be predicted by extrapolation from *in situ* studies of tree acclimation?
physiological responses to changes in prevailing ambient temperatures (i.e. seasonal acclimation and acclimation to climate warming are not equivalent)?

Leaf R often acclimates to changes in temperature, with increases in temperature resulting in reductions in R measured at a set temperature (reviewed by Slot & Kitajima, 2015), particularly because adjustments in the elevation of the short-term temperature response function appear to dominate acclimation responses (Type II acclimation; Atkin & Tjoelker, 2003). In most studies with trees, acclimation to warming results in partial homeostasis of R, with ambient and warm-grown trees often showing similar rates of R measured at the prevailing temperatures (Ow et al., 2010; Crous et al., 2011; Slot & Kitajima, 2015). The potential role of thermal acclimation of R in dampening the positive feedback between climate warming and atmospheric CO2 has stimulated new investigations of the pattern and extent of thermal acclimation across species, sites and biomes. For instance, by combining evidence for thermal acclimation of R to changes in prevailing ambient temperatures at local sites (i.e. Slot & Kitajima, 2015), with observed variation in R across a global temperature gradient (i.e. Atkin et al., 2015), Vanderwel et al. (2015) demonstrated that acclimation-over-time (i.e. seasonal adjustment) and acclimation-across-space (i.e. climate gradient) may result in similar changes in R. Yet, whether R in trees acclimates physiologically to seasonal temperature changes at a given site in a manner that is consistent under current and warmer climates has not been tested, with exception to one study in boreal and temperate tree species (Reich et al., 2016); however, such studies are important for clarifying the nature and magnitude of physiological plasticity to climate warming.

Moreover, it is unclear whether leaf A and R acclimate in a similar way to seasonal temperature changes and climate warming. Compared to thermal acclimation of R, studies in trees suggest that thermal acclimation of A may be somewhat constrained (Way & Sage, 2008; Ow et al., 2010; Way & Oren, 2010; Reich et al., 2015; Sendall et al., 2015). Thermal acclimation of A can occur via a change in the temperature optimum (Topt) of A, a change in A at the new growth temperature, or a change in photosynthetic capacity measured at a set temperature (Way & Yamori, 2014). In many cases, trees acclimate to higher growth temperatures by increasing the Topt of A (Battaglia et al., 1996; Gunderson et al., 2010; Way & Yamori, 2014), yet thermal acclimation does not necessarily increase photosynthetic performance in a warmer growth environment. In fact, acclimation to warmer growth temperatures can often result in downregulation of photosynthetic capacity measured at a set temperature (Way & Yamori, 2014; Ali et al., 2015; Way et al., 2015). Determining whether A and R acclimate in a similar way to climate warming and short-term seasonal temperature changes could advance, and potentially simplify, models which predict tree and forest responses to climate warming, and climate-C cycle feedbacks (Booth et al., 2012; Smith & Dukes, 2013; Vanderwel et al., 2015).

Comparative measurements of A and R at a set measurement temperature (e.g. 25°C) in relation to a change in growth-temperature can provide a direct measurement of the degree of acclimation (i.e. ‘set temperature method’; Mooney, 1963; Atkin et al., 2005). Thus, seasonal temperature acclimation can be quantified by repeatedly measuring A or R at a set temperature (e.g. 25°C) through time and examining the functional relationship between A or R at 25°C and the prevailing temperature as it varies seasonally (Atkin et al., 2005). Convergent acclimation of A and R to prevailing temperatures under current and warmer climatic conditions would be demonstrated if the relationship (linear or nonlinear) between A or R (at 25°C) and the prevailing growth temperature was equivalent among trees growing under current (i.e. ambient) and warmer climates (shown conceptually in Fig. 1a).

However, climate warming might alter the relationship between A or R (at 25°C) and prevailing temperatures, particularly if warming effects on A or R are manifested in changes in leaf structure or chemistry, such as leaf nitrogen (N) or total non-structural carbohydrate (TNC) concentrations. This may occur because total leaf N reflects total photosynthetic and respiratory enzyme content (Evans, 1989; Ryan et al., 1996; Reich et al., 1998; Atkin et al., 2015), and R is co-limited by the supply of TNC (i.e. substrates) and adenylate demand (Atkin & Tjoelker, 2003; Campbell et al., 2007; Tjoelker et al., 2009; O’Grady et al., 2010), and N and TNC are known to respond to changes in growth temperature (e.g. Farrar & Williams, 1991; Weih & Karlsson, 2001; Campbell et al., 2007). A negative relationship between leaf TNC concentrations and A might also be expected if increasing leaf TNC concentrations and low sink (i.e. growth, maintenance) capacity led to downregulation (reduction) of A, as observed in some elevated CO2 studies (Faria et al., 1996; Medlyn et al., 1999; Ainsworth et al., 2004). Thus, if climate warming alters leaf N or TNC concentrations, changes in the intercept of the relationship between A or R (at 25°C) and prevailing growth temperature might be expected (Fig. 1b). Alternatively, changes in the slope of this relationship could arise from warming effects on leaf N and TNC that varied with prevailing growth temperature, such that climate warming effects differ seasonally between cool and warm time periods (Fig. 1c). Furthermore, given that carbohydrates are products of \( \text{A} \) and substrates for tissue formation (i.e. growth) and maintenance processes (including \( \text{R} \), associations between seasonal changes in leaf TNC concentrations, tree growth rate, and the ratio of \( \text{R} \) to \( \text{A} \), could help to explain whether source–sink dynamics influence patterns of physiological acclimation to temperature (Körner, 2003; Bansal & Germino, 2008).

Here, we grew 12 Eucalyptus tereticornis trees in the field inside large whole-tree chambers under two temperature treatments: one tracking ambient air temperature and another tracking ambient air temperature + 3°C warming. Each month throughout a 14-month period, we measured leaf-level rates of light saturated A and night-time R at prevailing ambient temperatures, as well as R and light- and CO2-saturated A at a set temperature of 25°C to assess respiratory and photosynthetic temperature acclimation. We also determined leaf N and TNC concentrations of the measured leaves. We then tested whether A and R acclimate to seasonal temperature changes in a manner that is consistent between ambient and warmed temperature conditions, and whether
changes in leaf N or TNC with warming or seasonal temperature changes were associated with patterns of thermal acclimation. Lastly, we assessed potential source–sink effects on patterns of thermal acclimation by examining relationships between tree growth rate, leaf TNC concentrations, and the balance between leaf R and A measured at prevailing ambient temperatures.

 материалов и методы

студийной и экспериментальной работы

это исследование проводилось в эксперименте в Хаувсбери, НСВ, Австралия (33°36’38.92”S, 150°44’27.75”E). местный климат теплый-субтропический, с летним (ноябрь–февраль) преобладанием и среднегодовым приходом сухого в 800 мм. среднегодовая температура равна 17°C. максимальная межгодовая температура в январе составляет 29.6°C, и минимальная межгодовая температура в июле 3.6°C (австралийский бюро meteorology). почвы в clarendon formation (chromosol), характеризуются песчаными сосновыми почвами с низким содержанием органического вещества (0.7%), и средним содержанием минерального органического вещества.

стиль изложения: научная работа

в этом исследовании использовалось 12 больших цилиндрических алюминиевых рамных одноместных камер (Barton et al., 2010, для деталей), которые были модифицированы для обеспечения режима температурного контроля, как описано в Crous et al. (2013). кратко, каждый камера была 3.25 м в ширину и 9 м в высоту и была покрыта тонким слоем self-cleaning ethylene-tetrafluoroethylene copolymer film, which transmits both UV and visible light (>90%) (F-Clean; AGC Chemicals Pty Ltd, Singapore). фильм плос и пропускает облучение и обеспечивают организованное корневое пространство.

отсутствие влияния на листьевые признаки

могло привести к положительному или отрицательному отношению между

могло привести к положительной или отрицательной зависимости между

могло привести к положительной или отрицательной зависимости между

могло привести к положительной или отрицательной зависимости между
On 26 November 2012, the seedlings were transplanted into 25-l pots filled with soil collected from the study site. On 5 December 2012, six seedlings were placed into each chamber. The temperature treatments began on 12 December 2012. Among the seedlings growing in the ambient and warmed chambers ($n=6$), six of each temperature treatment were selected which were of similar size (based on seedling height and basal diameter) and free of abnormalities, and one seedling was planted within each chamber on 12 March 2013. Tree height and diameter, starting at 65 cm above the ground and continuing at 30 cm intervals, were measured every 14 d throughout the experiment. Total bole volume ($m^3$) was estimated at each measurement point by calculating the volume of each stem section based on a frustum of a cone, and summing the volume of each stem section. We assumed that the tree stem was cylindrical from the ground to 65-cm height. Absolute bole volume growth rate ($cm^3 \text{ d}^{-1}$) was calculated as the absolute difference in volume across each measurement interval.

Leaf-level gas exchange, nitrogen and nonstructural carbohydrates

The effects of the temperature treatment (ambient or warmed) and seasonal temperature changes on leaf physiology were examined by measuring leaf-level light saturated net photosynthesis ($A_{sat}$) and night-time dark respiration ($R$) at prevailing temperatures on 11 d, c. 30 d apart, between May 2013 and April 2014. Physiological acclimation to climate warming and seasonal temperature changes was determined using the set-temperature method (Atkin et al., 2005) where light- and CO2-saturated net photosynthesis and night-time $R$ were measured at a common temperature ($25^\circ\text{C}$) on the same leaves at 10 of the 11 time points (June 2013–April 2014). The following protocol was followed on each measurement day. To monitor tree water status, predawn and midday leaf xylem water potential ($\Psi_{pd}$ and $\Psi_{md}$) were measured on 1–3 mature, fully expanded upper canopy leaves per tree using a Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA). $\Psi_{pd}$ was measured roughly 30 min before sunrise and $\Psi_{md}$ was measured at 12:30 h ($\pm$ 30 min).

Measurements of $A_{sat}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance to water vapour ($g_{s} \text{ mmol m}^{-2} \text{s}^{-1}$) were measured between 09:30 and 12:00 h on three sun-exposed, mature, fully expanded leaves per tree, located on the outer edge of the upper portion of the tree canopy. Three LI-6400XT portable photosynthesis systems (Li-Cor Inc., Lincoln, NE, USA) were operated in separate whole-tree chambers. For all measurements, reference [CO2] was adjusted to achieve a constant chamber [CO2] of 400 $\pm$ 5 $\mu\text{mol l}^{-1}$. Light intensity within the leaf chamber was maintained at 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD) using the red/blue LED light source. Flow rate was held constant at 500 $\mu\text{mol s}^{-1}$. Water vapour inside the leaf chamber was not scrubbed so that relative humidity inside the cuvette approximated ambient conditions inside the whole-tree chamber. We maintained a 3°C difference in average leaf temperature ($T_{leaf}$) between warmed and ambient treatments by controlling the LI-6400XT leaf chamber block temperature. Immediately following measurements of $A_{sat}$ under ambient $T_{air}$, $T_{leaf}$ was set at 25°C and the rate of light- and CO2-saturated net photosynthesis was measured ($A_{25}^\text{max}$; 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD, 1800 $\mu\text{mol CO}_2 \text{ mol}^{-1}$). When a $T_{leaf}$ of 25°C could not be achieved with the LI-6400XT temperature controls, the $T_{air}$ of the whole-tree chamber was temporarily increased or decreased to allow for gas exchange measurements at 25°C.

We measured $A_{max}^\text{25}$ because $A_{max}^\text{25}$ represents the maximum $A_{sat}$ derived from a photosynthetic CO2 response ($A-C_i$) curve, and therefore provides a rapid assessment of photosynthetic capacity that is insensitive to low or variable intercellular [CO2] ($C_i$) associated with partial stomatal closure (e.g. under high VPD). Indeed, we measured $A-C_i$ curves at 25°C on similar leaves at eight time points throughout the study period by measuring $A_{sat}$ at 12 reference [CO2] between 50 and 1800 $\mu\text{mol mol}^{-1}$. Light intensity, flow rate and humidity conditions were maintained as for measurements of $A_{sat}$. The biochemical model of photosynthesis (Farquhar et al., 1980) was fitted using PROC NLIN in SAS v.9.3 (SAS Institute Inc., Cary, NC, USA), and was used to parameterize each $A-C_i$ curve. The model estimates the maximum rate of Rubisco carboxylation ($V_{cmax}^\text{25}$; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the maximum rate of electron transport for RuBP regeneration under saturating light ($J_{max}^\text{25}$; $\mu\text{mol m}^{-2} \text{s}^{-1}$). Leaf mesophyll conductance to CO2 was not estimated, thus $V_{cmax}^\text{25}$ and $J_{max}^\text{25}$ are apparent values. Importantly, we found that $A_{max}^\text{25}$ derived from these $A-C_i$ curves showed a strong positive relationship with $J_{max}^\text{25}$ (Fig. S2; $r^2=0.94$) and a positive, albeit weaker, relationship with $V_{cmax}^\text{25}$ ($r^2=0.51$) likely arising from the correlation between $J_{max}^\text{25}$ and $V_{cmax}^\text{25}$. Furthermore, short-term temperature response curves of $A_{max}$ (i.e. $A_{max}^\text{25}$ vs $T_{leaf}$) measured in three seasons (spring, summer and autumn) revealed no change in the temperature optimum of $A_{max}$ with climate warming or season (Fig. S3). Instead, the entire short-term temperature response curve of $A_{max}$ shifted downward as seasonal temperatures increased (or upward as seasonal temperatures decreased), as well as with experimental warming (Fig. S3). Thus, in these trees, repeated measures of $A_{max}^\text{25}$ provided a robust metric of acclimation of photosynthetic capacity.

Measurements of night-time leaf $R$ were conducted at the prevailing chamber $T_{air}$ and were taken on the evening following measurements of $A_{sat}$ on the same leaves. Measurements of $R$ were made on excised leaves and collection of leaves began 2 h after sunset. Sampling order occurred randomly among the 12 chambers within and across dates. The entire leaf was placed in a large gas exchange chamber (LI-6400-22L; Li-Cor) to increase the CO2 differentials and more accurately determine CO2 efflux rates with minimal CO2 leakage. For measurements under pre-existing conditions, the block temperature was maintained at ambient night-time $T_{air}$ and reference [CO2] was fixed at 400 $\mu\text{mol mol}^{-1}$. Leaves were then moved to a temperature-controlled trailer at the site set to c. 25°C. About 20 min after being measured at the prevailing night-time ambient $T_{air}$, leaves were placed in another LI-6400-22L chamber and $R$ was measured at 25°C. In the same study, Drake et al. (2016)
demonstrated that climate warming alters the short-term instantaneous temperature response of leaf $R$ by reducing rates of $R$ at a set temperature without changing the temperature sensitivity (i.e. activation energy, $Q_{10}$) of $R$. Thus, measurements of leaf $R$ at a set temperature of 25°C provide a direct measure of the degree of respiratory temperature acclimation.

Following measurements of leaf $R$, leaf area (cm$^2$) was determined with a leaf area meter (LI-3100C; Li-Cor). Leaves were dried to a constant mass at 70°C, and ground into a fine powder using a ball mill, stored under desiccation, and leaf N (mg g$^{-1}$) was determined using a combustion elemental analyser (TruSpec Micro, Leco, St Joseph, MI, USA). Leaf dry mass per unit area (LMA, g m$^{-2}$) was calculated and used to express leaf N on an area basis ($N_{area}$ g N m$^{-2}$).

The ground leaf material was used to determine concentrations of leaf nonstructural carbohydrates (soluble sugars, starch) following Tissue & Wright (1995). Comparison of soluble sugar and starch concentrations measured using different techniques is not advisable between studies (Quentin et al., 2015), yet comparisons within a single study are still appropriate. Total nonstructural carbohydrate concentrations (TNC, mg g$^{-1}$) were calculated by summing concentrations of soluble sugars and starch. Because carbohydrates can represent a signification but variable proportion of leaf mass, thereby influencing apparent values of LMA and $N_{area}$, we also calculated TNC-free LMA and TNC-free $N_{area}$. TNC-free LMA was calculated as:

$$TNC \text{- free LMA} = \frac{LM - (LM \times TNC)}{LA} \quad \text{Eqn 1}$$

(LM, dry mass (g) of the sampled leaf; TNC, leaf TNC concentration in g TNC g$^{-1}$ leaf dry mass; LA, leaf area (m$^{-2}$) of the sampled leaf). TNC-free $N_{area}$ was calculated as:

$$TNC \text{- free } N_{area} = \frac{(N_{TNC} \times LM)}{LA} \quad \text{Eqn 2}$$

($N$, leaf N in g N g$^{-1}$ leaf dry mass).

Statistical analysis

Statistical analyses were conducted in SAS v.9.3 (SAS Institute). Linear mixed-effect models were used to test the effects of time (i.e. date), temperature treatment (ambient or warmed) and time × temperature treatment interactions on each response variable. Chamber within-treatment (10 degrees of freedom) and time × chamber within-treatment effects were considered as random effects. LMA, $N_{area}$, g, and R data were log-transformed to fulfil assumptions of normality. Linear regression was used to test associations between treatment mean $A_{max}$, $V_{cmax}$, $J_{max}$ and $R$ measured at 25°C at each sampling date, and the mean daily minimum, mean daily and mean daily maximum $T_{air}$ of the preceding 3, 5, 10, 15 and 30 d. Significant regression relationships between rates at 25°C and preceding temperature conditions provided evidence of physiological acclimation to seasonal temperature changes.

Analysis of covariance (ANCOVA) was used to test for temperature treatment effects on the intercept and slope parameters describing the relationship between $A_{max}$, $V_{cmax}$, $J_{max}$ and $R$ (at 25°C), and prevailing $T_{air}$. ANCOVA also was used to test for temperature treatment effects on the association between sampling date mean values of leaf N and TNC and $A_{max}$ and $R$ measured at 25°C. Associations between sampling date mean values of LMA, N, and TNC and prevailing $T_{air}$ were not modelled because we had no a priori expectation about the nature of these associations. The long-term temperature response of in situ $A_{sat}$ was fitted using a parabola function (Battaglia et al., 1996), providing the $T_{opt}$ of $A_{sat}$ ($T_{leaf}$ at maximum $A_{sat}$). The long-term temperature response of in situ $R$ was fitted using an exponential model with a fixed $Q_{10}$ (parameter describing increase in $R$ given a 10°C increase in temperature) (e.g. Tjoelker et al., 1999). All tests of statistical significance were conducted at an alpha of 0.05.

Results

$A_{max}$ and R measured at 25°C

On average, the warming treatment (+ 3°C) significantly reduced rates of $A_{max}$ by 8.4% (Tables 1, 2), providing direct evidence for photosynthetic acclimation to climate warming. $A_{max}$ also varied over time, decreasing as mean daily $T_{air}$ increased (Fig. 2b). The 3-d mean daily $T_{air}$ was strongly negatively correlated with temporal variation in $A_{max}$ (Fig. 2b), although 5-, 10-, 15- and 30-d mean daily $T_{air}$ were also correlated with a decline in $A_{max}$ (Table S1). Importantly, the relationship between $A_{max}$ and 3-d mean daily $T_{air}$ was nonlinear; $A_{max}$ decreased at an increasing rate with increasing mean daily $T_{air}$ (Fig. 2b). A polynomial model was fitted to this relationship and warming did not affect the intercept ($P = 0.27$), slope ($P = 0.35$) or quadratic term ($P = 0.38$). Thus, the strength of thermal acclimation of photosynthetic capacity to seasonal temperature changes was dependent upon prevailing growth temperature, but was equivalent for trees grown under current and warmer climates (Fig. 2b). $J_{max}$ was reduced by 10.4% with climate warming (Tables 1, 2), varied over time (Table 1; Fig. 2e) and decreased with increasing mean daily $T_{air}$ in a manner that was consistent across treatments (Fig. 2f). Alternatively, warming effects on $V_{cmax}$ varied over time (Table 1; Fig. 2c), and no relationship between $V_{cmax}$ and mean daily $T_{air}$ was observed ($P = 0.50$).

The warming treatment also reduced rates of $R$ (area or mass basis) measured at 25°C (Tables 1, 2). On average, warming reduced $R_{area}$ by 21.1% (Table 2), providing evidence for respiratory acclimation to climate warming. Leaf $R$ (area or mass basis) measured at 25°C also varied over time (Table 1; Fig. 2g) and decreased as mean daily $T_{air}$ increased (Figs 2h, S4b). Warming did not affect the intercept or slope of the relationship between $R_{area}$ (intercept $P = 0.74$; slope $P = 0.81$) and mean daily $T_{air}$ (Fig. 2d), indicating that leaf respiration acclimated to seasonal temperature changes, and did so in an equivalent manner in ambient- and warm-grown trees.
Table 1  Linear mixed-effect model analysis of variance of time (i.e. measurement date), temperature treatment (ambient, ambient + 3°C), and time × temperature treatment effects on leaf photosynthesis and respiration (at a set temperature and prevailing temperature), as well as leaf carbohydrate concentration, leaf mass per unit area, and leaf nitrogen concentration per unit area in Eucalyptus tereticornis

<table>
<thead>
<tr>
<th>Time</th>
<th>Treatment</th>
<th>Time × treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf measurements (set temperature, 25°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( A_{\text{max}}^{25} )</td>
<td>9.77</td>
<td>7.7***</td>
</tr>
<tr>
<td>( V_{\text{max}}^{25} )</td>
<td>7.58</td>
<td>10.7***</td>
</tr>
<tr>
<td>( j_{\text{max}}^{25} )</td>
<td>7.58</td>
<td>9.8***</td>
</tr>
<tr>
<td>( R_{\text{area}}^{25} )</td>
<td>9.78</td>
<td>57.1***</td>
</tr>
<tr>
<td>( R_{\text{mass}}^{25} )</td>
<td>9.78</td>
<td>16.1***</td>
</tr>
</tbody>
</table>

| Leaf measurements (prevailing temperature) | | |
| \( A_{\text{sat}} \) | 11.92 | 5.4*** | 1.10 | 1.5 | 11.92 | 0.8 |
| \( R_{\text{area}} \) | 10.88 | 10.1*** | 1.10 | 0.1 | 10.88 | 1.5 |
| \( R_{\text{mass}} \) | 10.88 | 11.2*** | 1.10 | 0.7 | 10.88 | 0.8 |
| Starch | 10.88 | 17.1*** | 1.10 | 0.02 | 10.88 | 1.1 |
| Soluble sugars | 10.88 | 31.4*** | 1.10 | 4.1 | 10.88 | 1.5 |
| TNC | 10.88 | 31.2*** | 1.10 | 0.05 | 10.88 | 1.3 |
| LMA | 10.88 | 8.6*** | 1.10 | 1.0 | 10.88 | 0.7 |
| TNC-free LMA | 10.88 | 5.3*** | 1.10 | 1.9 | 10.88 | 0.9 |
| Narea | 10.88 | 6.7*** | 1.10 | 6.2* | 10.88 | 1.7 |
| TNC-free Narea | 10.88 | 3.6** | 1.10 | 3.6 | 10.88 | 1.9 |

Numerator and denominator degree of freedom (df) and \( F \)-values are presented for each variable and effect. \( F \)-values significant at: *** \( P < 0.001; ** \( P < 0.01; * \( P < 0.05 \). Variable descriptions: \( A_{\text{max}}^{25} \), light- and CO₂-saturated net photosynthesis measured at 25°C; \( V_{\text{max}}^{25} \), maximum rate of Rubisco carboxylation measured at 25°C; \( j_{\text{max}}^{25} \), maximum rate of electron transport for RuBP regeneration measured at 25°C; \( R_{\text{area}}^{25} \), area-based dark respiration measured at 25°C; \( R_{\text{mass}}^{25} \), mass-based dark respiration measured at 25°C; \( A_{\text{sat}} \), light-saturated net photosynthesis; \( R_{\text{area}} \), area-based dark respiration; \( R_{\text{mass}} \), mass-based dark respiration; TNC, total nonstructural carbohydrate concentration (starch + soluble sugars); LMA, leaf dry mass per unit leaf area; TNC-free LMA, TNC-free leaf dry mass per unit leaf area; \( N_{\text{area}} \), leaf N per unit leaf area; TNC-free \( N_{\text{area}} \), TNC-free leaf N per unit leaf area.

\( A_{\text{sat}} \) and \( R \) measured at prevailing ambient temperatures

Rates of \( A_{\text{sat}} \) and \( R \) (area or mass-based) measured at the prevailing ambient \( T_{\text{air}} \) varied over time, but did not differ between ambient and warmed trees (Tables 1, 2; Figs 3a,c, S4). Notably, across all measurements there was a strong positive relationship between \( A_{\text{sat}} \) and \( A_{\text{max}} \) measured at 25°C, indicating that light-saturated photosynthetic rates were higher when photosynthetic capacity was higher (Fig. S5). Across aggregate temperature measurements and measurement dates, \( A_{\text{sat}} \) showed a broad relationship with prevailing \( T_{\text{leaf}} \) with an apparent \( T_{\text{opt}} \) of 22°C (Fig. 3b). Across treatments and measurement dates, \( A_{\text{sat}} \) increased as prevailing \( T_{\text{leaf}} \) increased (Fig. 3d). The realized \( Q_{10} \) value describing the long-term acclimated temperature response of \( R_{\text{area}} \) to prevailing \( T_{\text{leaf}} \) was 1.22. The ratio of \( R_{\text{area}} \) to \( A_{\text{sat}} \) (i.e. \( R_{\text{area}}/A_{\text{sat}} \)) varied modestly when mean daily \( T_{\text{air}} \) (24-h) was between 10 and 20°C, but increased when mean daily \( T_{\text{air}} \) exceeded 20°C (Fig. 4b). \( \Psi_{pd} \) averaged \(-0.24 \pm 0.11 \) (SD, MPa) through time, did not differ between warming treatments (\( P = 0.60 \)) and was not associated with variation in \( A_{\text{sat}} \) (data not shown).

Leaf carbohydrates, LMA and leaf N

Warming treatment had no effect on leaf starch, soluble sugars or TNC concentrations (Table 1), but all three traits varied over time (Table 1; Fig. 5a–c). Starch and TNC concentrations were highest during cool periods (TNC ≥ 300 mg g⁻¹) and declined as mean daily \( T_{\text{air}} \) increased (Fig. 5b,d). Leaf soluble sugar concentrations remained relatively constant (≤ 100 mg g⁻¹) when mean daily \( T_{\text{air}} \) was between 10 and 20°C, but decreased when mean daily \( T_{\text{air}} \) exceeded 20°C (Fig. 5f).

LMA was not affected by warming, but varied over time (Table 1; Fig. S6a) and with changes in mean daily \( T_{\text{air}} \) (Fig. S6b), as did \( N_{\text{area}} \) (Fig. S6c,d). However, TNC-free LMA and TNC-free \( N_{\text{area}} \) were not affected by warming (Table 1), and although both traits varied over time (Table 1; Fig. S6e,g), there was no relationship between mean daily \( T_{\text{air}} \) and TNC-free LMA and TNC-free \( N_{\text{area}} \) (Fig. S6h). Thus, both LMA and \( N_{\text{area}} \) showed little variation with changes in mean daily \( T_{\text{air}} \) or in response to warming after accounting for changes in leaf mass associated with leaf carbohydrates.

Associations between TNC, leaf N, photosynthetic capacity and respiration

If temperature acclimation of \( A_{\text{max}}^{25} \) and \( R_{\text{area}}^{25} \) was influenced by variation in leaf N or TNC concentrations related to seasonal and warming treatment effects, we would expect to have observed significant relationships between physiological measurements (e.g. \( A_{\text{max}}^{25} \)) and leaf N and TNC concentrations that were similar across ambient and warmed treatments. Across treatments, \( A_{\text{max}}^{25} \) showed a weak positive relationship with TNC-free \( N_{\text{area}} \) (Fig. 6a), whereas \( R_{\text{area}}^{25} \) showed no relationship with TNC-free \( N_{\text{area}} \) (Fig. 6b). By contrast, strong positive relationships were found between \( A_{\text{max}}^{25} \) and TNC, and \( R_{\text{area}}^{25} \) and TNC (Fig. 6c, d). Warming reduced the intercept of the relationship between \( A_{\text{max}}^{25} \) and TNC (\( P = 0.03 \)), but not the slope (\( P = 0.14 \); Fig. 6c). When \( A_{\text{max}}^{25} \) and \( R_{\text{area}}^{25} \) were expressed per unit leaf N (i.e.
Leaf traits that responded significantly to warming (i.e. % change): *** P<0.001; * P≤0.05. Variable descriptions: A<sub>max</sub><sup>25</sup>, light- and CO<sub>2</sub>-saturated net photosynthesis measured at 25°C; V<sub>max</sub><sup>25</sup>, maximum rate of Rubisco carboxylation measured at 25°C; J<sub>max</sub><sup>25</sup>, maximum rate of electron transport for RuBP regeneration measured at 25°C; R<sub>area</sub><sup>25</sup>, area-based dark respiration measured at 25°C; R<sub>mass</sub><sup>25</sup>, mass-based dark respiration measured at 25°C; A<sub>sat</sub>, light-saturated net photosynthesis; R<sub>area</sub>, area-based dark respiration; R<sub>mass</sub>, mass-based dark respiration; TNC, total nonstructural carbohydrate concentration (starch + soluble sugars); LMA, leaf dry mass per unit leaf area; TNC-free LMA, TNC-free leaf dry mass per unit leaf area; N<sub>area</sub>, leaf N per unit leaf area; TNC-free N<sub>area</sub>, TNC-free leaf N per unit leaf area.

A<sub>max</sub><sup>N</sup>, R<sub>area</sub><sup>N</sup> both variables increased with increasing leaf TNC (Fig. 6c,d), and warming did not affect the intercept or slope of either relationship (all P-values > 0.24). Thus, acclimation of photosynthetic capacity and respiration were associated primarily with variation in leaf TNC, rather than leaf N.

Associations between leaf TNC, tree growth rate and leaf C balance

Averaged over time, climate warming increased tree bole volume (P=0.03) (Fig. 7a). To determine whether source–sink dynamics might explain the seasonal variation in leaf TNC concentrations that were strongly associated with physiological acclimation to temperature, we tested relationships between leaf TNC concentrations, bole volume absolute growth rate, and the ratio of in situ R to A. Across treatments, seasonal declines in leaf TNC concentrations were correlated with an increase in bole volume growth rate (Fig. 7b). Likewise, leaf TNC concentrations declined as leaf R<sub>area</sub>/A<sub>sat</sub> increased, indicating that leaf TNC concentrations increased when respiration was low relative to photosynthesis (Fig. 7c).

Discussion

We grew trees of a widespread broadleaved evergreen species, *Eucalyptus tereticornis*, in the field inside large whole-tree chambers under ambient and warmed temperature treatments, and tested whether acclimation of leaf photosynthesis (A) and dark respiration (R) to seasonal temperature change and climate warming mirror each other in a consistent predictable manner. We also tested whether temperature effects on leaf nitrogen (N) or total nonstructural carbohydrate (TNC) concentrations were associated with thermal acclimation of A and R. We found that acclimation of A and R to seasonal temperature change was equivalent to physiological acclimation to climate warming (e.g. Fig. 1a was supported). Moreover, changes in leaf TNC concentrations (but not leaf N) were related to thermal acclimation of both processes, indicating a connection between photosynthesis, carbohydrate availability and respiration. The convergence of physiological acclimation to seasonal temperature changes and climate warming could potentially simplify predictions of tree and forest responses to atmospheric warming, and climate–carbon (C) cycle feedbacks.

Patterns and strength of acclimation of CO<sub>2</sub> exchange rates to temperature

Emerging evidence for thermal acclimation of leaf physiology has stimulated new analyses of its pattern and extent. For instance,
The response of temperature optimum, function is fitted to the relationship between $R_{\text{area}}$ and plotted through time (a, c, e; day/month/year) and in relation to mean daily (3-d mean) air temperature ($T_{\text{mean}}$; b, d). The fitted parameters were: $R_{\text{area}} = R_{15}Q_{10}^{10\left(T_{\text{mean}} - 15\right)/10}$, where $R_{15}$ is $R_{\text{area}}$ at a reference $T_{\text{mean}}$ of 15°C and $Q_{10}$ describes the change in $R_{\text{area}}$ given a 10°C change in $T_{\text{mean}}$. The fitted parameters were: $R_{15} = 0.46$, $Q_{10} = 1.22$.

Fig. 4 Mean values (± SE) of the ratio of in situ $R_{\text{area}}$ to in situ $A_{\text{sat}}$ ($R_{\text{area}}/A_{\text{sat}}$), both measured at mean night and day leaf temperatures in *Eucalyptus tereticornis*, plotted over time (day/month/year) (a) and in relation to prevailing mean daily (24-h) air temperature (b, $T_{\text{air}}$). A cubic function is fitted to the relationship between $R_{\text{area}}/A_{\text{sat}}$ and 24-h mean $T_{\text{air}}$ in (b): $y = -0.039 + 0.012x - 0.0007x^2 + 0.0000153x^3$ ($R^2 = 0.80$, $P < 0.0001$).

Fig. 3 Mean (± SE) rates of in situ light-saturated net photosynthesis ($A_{\text{sat}}$) and corresponding night-time dark respiration ($R_{\text{sat}}$) measured in *Eucalyptus tereticornis* and plotted through time (a, c, day/month/year) and in relation to mean measured (day or night) leaf temperatures ($T_{\text{leaf}}$; b, d). The response of $A_{\text{sat}}$ to prevailing $T_{\text{leaf}}$ in (b) was fitted using a parabola function: $A_{\text{sat}} = A_{\text{opt}} - b(T_{\text{leaf}} - T_{\text{opt}})^2$, where $A_{\text{opt}}$ is $A_{\text{sat}}$ at the temperature optimum, $T_{\text{opt}}$, and the parameter $b$ describes the spread of the function. The fitted parameters were: $A_{\text{opt}} = 18.5$, $T_{\text{opt}} = 22.0$, and $b = 0.045$. The response of $R_{\text{area}}$ to prevailing $T_{\text{leaf}}$ in (d) was fitted using the equation: $R_{\text{area}} = R_{15}Q_{10}^{10\left(T_{\text{leaf}} - 15\right)/10}$, where $R_{15}$ is $R_{\text{area}}$ at a reference $T_{\text{leaf}}$ of 15°C and $Q_{10}$ describes the change in $R_{\text{area}}$ given a 10°C change in $T_{\text{leaf}}$. The fitted parameters were: $R_{15} = 0.46$, $Q_{10} = 1.22$.

using data from Slot & Kitajima (2015) and Atkin *et al.* (2015), Vanderwel *et al.* (2015) found that acclimation-over-time and acclimation-across-space may result in similar changes in leaf $R$ in response to changes in temperature. However, this conclusion was based on a global survey across sites and species, rather than a test of whether physiological acclimation to climate warming and seasonal temperature changes are equivalent within sites, and for a given species. We directly tested this question and found that acclimation of $A$ and $R$ to seasonal temperature changes at a given site was nearly identical to acclimation to experimental temperature change of a similar magnitude. Recent work with northern high-latitude tree species found similar patterns for respiration (Reich *et al.*, 2016), suggesting this may be a common phenomenon.

The magnitude of physiological acclimation to seasonal temperature changes was also particularly notable: $R_{\text{area}}^{25}$ declined by 43% with a 10°C increase in 3-d mean daily ambient air temperature ($T_{\text{mean}}$). This magnitude of acclimation was similar to and on average stronger than acclimation of $R$ previously observed over a wide range of tree species (Atkin *et al.*, 2000; Bolstad *et al.*, 2003; Lee *et al.*, 2005; Bruhn *et al.*, 2007; Tjoelker *et al.*, 2008; O’Grady *et al.*, 2010; Crous *et al.*, 2011). The response of in situ $R_{\text{area}}$ measured under prevailing ambient temperatures also demonstrated the strength of seasonal acclimation. The $Q_{10}$ value describing the long-term temperature response of $R$ on a leaf area basis ($R_{\text{area}}$)(1.22) was substantially lower than the measured $Q_{10}$ value of 2.3 in this study (Drake *et al.*, 2016), or a $Q_{10}$ value of 2.0, which is typical of the short-term temperature response of $R$ over this temperature range (Tjoelker *et al.*, 1999; Atkin & Tjoelker, 2003), and is commonly used in terrestrial biosphere models (Whithers *et al.*, 2005; King *et al.*, 2006; Atkin *et al.*, 2008). This long-term $Q_{10}$ for $R_{\text{area}}$ is remarkably similar to the long-term $Q_{10}$ value of 1.2 found by Reich *et al.* (2016), but is significantly lower than literature-based $Q_{10}$ values used in a global model of leaf $R$ which incorporates acclimation over time and space (i.e. $Q_{10} = 1.45$ to 1.65; Slot & Kitajima, 2015; Vanderwel *et al.*, 2015). The strong reduction in the in situ temperature sensitivity of $R$ over longer time periods arises directly from thermal acclimation of $R$, resulting in lower-than-expected $R$ during warm seasons and higher-than-expected $R$ during cool seasons compared to the instantaneous short-term temperature response of $R$ (see Atkin *et al.*, 2008). This finding indicates that acclimation may strongly reduce seasonal variation in $R$ and thus dampen the long-term temperature response of leaf $R$ (Atkin *et al.*, 2008; Tjoelker *et al.*, 2008, 2009; Vanderwel *et al.*, 2015).

We also observed seasonal acclimation of photosynthetic capacity, but unlike the marked linear decline in $R_{\text{area}}$ at 25°C
with increasing mean daily air temperature ($T_{\text{air}}$), the response of light- and CO$_2$-saturated net photosynthesis at 25°C ($A_{\text{max}}^{25}$) was clearly nonlinear. $A_{\text{max}}^{25}$ appeared unaffected when mean daily $T_{\text{air}}$ was low to moderate ($\leq 15^\circ$C), but declined at higher temperatures. $A_{\text{max}}$ was largely reflective of maximum rate of electron transport for RuBP regeneration under saturating light ($J_{\text{max}}^{25}$) limitations for the trees in our study, and indeed $J_{\text{max}}^{25}$ also declined as mean daily $T_{\text{air}}$ increased, and did so consistently across ambient and warmed temperature treatments. These findings could indicate that higher summer temperatures reduce electron transport capacity for RuBP regeneration (Sage & Kubien, 2007), and ambient and warmed trees may show similar reductions in RuBP regeneration capacity as prevailing growth temperatures increase. Our results also support the idea that $J_{\text{max}}^{25}$ is more sensitive to high temperature than carboxylation capacity (i.e. $V_{\text{cmax}}^{25}$; Schrader et al., 2004; Wise et al., 2004; Sage & Kubien, 2007), presumably because RuBP regeneration capacity is dependent upon membrane stability. Our findings are notable considering that evidence for thermal acclimation of leaf photosynthetic capacity to warming and seasonal temperature changes is rather mixed (Kattge & Knorr, 2007; Ow et al., 2008, 2010; Way & Sage, 2008; Dillaway & Kruger, 2010; Gunderson et al., 2010; Sendall et al., 2015). In comparison to our study, Medlyn et al. (2002) found that $V_{\text{cmax}}^{25}$ and $J_{\text{max}}^{25}$ declined linearly as the mean minimum temperature of the preceding month increased in Pinus pinaster. In a global analysis of variability in
C₃ plant photosynthetic capacity, Ali et al. (2015) also found that $V_{\text{cmax}}^{25}$ and $J_{\text{max}}^{25}$ declined with increasing growth temperature. Further empirical work across different species and biomes is needed to improve our understanding of the pattern and magnitude of seasonal acclimation of photosynthetic capacity.

Interestingly, we found that both $R_{\text{area}}^{25}$ and $A_{\text{max}}^{25}$ were negatively correlated with the short- and long-term mean daily temperature (shown in Table S1). The negative correlation with the 3-d mean daily $T_{\text{air}}$ suggests that respiratory physiology and photosynthetic capacity may adjust dynamically to recent changes in temperature. This is interesting considering that we measured mature, fully expanded outer canopy leaves which had likely developed under temperature conditions that were different from the preceding 3 d, and in woody plant species leaves developed under a particular growth temperature generally have less capacity to acclimate to a new growth temperature than newly formed leaves (Atkin & Tjoelker, 2003; Campbell et al., 2007; Slot & Kitajima, 2015). However, Eucalyptus tereticornis continuously produces new leaves that in our study developed under a range of growth temperature conditions across seasons. Thus, our frequent measurement interval (c. 30 d) likely captured the full magnitude of temperature acclimation, potentially encompassing both short-term (days) and long-term adjustments in physiology underpinning leaf CO₂ exchange rates.

Our observations of thermal acclimation at the leaf scale also align with observations of thermal acclimation at the canopy scale reported in Drake et al. (2016) in the same trees. Drake et al. (2016) found that acclimation to climate warming resulted in similar rates of in situ canopy $R$ among ambient- and warm-grown trees, and the ratio of canopy-scale $R$ to canopy-scale $A$ was equivalent for ambient- and warm-grown trees across all conditions apart from exceptionally hot time-periods. Both studies indicate that acclimation may result in a near-constant ratio of $R$ to $A$ at low to moderate temperatures, but the proportion of fixed C that is respired may still increase during hot conditions. Thus, thermal acclimation may limit respiratory CO₂ fluxes with warming, but a greater number of hot days may result in a positive feedback between climate warming and atmospheric CO₂.

Associations between nonstructural carbohydrates and physiological acclimation to temperature

We examined whether changes in leaf N or TNC with warming or seasonal temperature changes can explain observed patterns of thermal acclimation of photosynthesis and respiration. Variation in leaf $R$ is expected to be positively associated with leaf N and TNC concentrations given that total leaf N, in part, reflects total respiratory enzyme as well as protein content and maintenance costs, and carbohydrates are substrates for respiration, although adenylate demand may co-limit respiratory capacity (Tjoelker et al., 1999, 2008, 2009; Atkin et al., 2000, 2015; Drake et al., 2008). We found that seasonal acclimation of $R$ (on an area and N-basis, i.e. $R_{\text{area}}^{25}$, $R_{\text{N}}^{25}$) was linked to changes in leaf TNC, but total leaf N (i.e. TNC-free $N_{\text{area}}$) did not change with warming and was not associated with seasonal variation in $R$ at 25°C. The positive association between leaf TNC concentrations and $R$ at 25°C was likely co-regulated by temperature limitations on metabolic processing of TNC for growth, maintenance, and $R$ (Atkin & Tjoelker, 2003), but also the supply of TNC fixed through photosynthesis (Kozlowski, 1992; Dewar et al., 1999; Bansal & Germino, 2009). In our study, tree growth rates and in situ $R$ were generally lower during cool periods, whereas in situ $A$ was generally high, which may explain the increase in leaf TNC concentrations during cool periods, and the positive link between carbohydrates and $R$ at 25°C.

In comparison to our study, Crous et al. (2011) found lower starch concentrations in summer than in winter in Eucalyptus saligna, yet variation in $R$ was not clearly linked with leaf N or TNC concentrations. Yet, O’Grady et al. (2010) found positive associations between seasonal changes in leaf $R$, and leaf N and...
TNC in *Eucalyptus globulus*. In our study, average leaf N was relatively high (2.5% of leaf dry mass or 3.2% of TNC-free leaf dry mass; data not shown) and showed little seasonal variation and no effect of warming treatment, whereas leaf TNC concentrations showed substantial seasonal variation. This differential temporal variation may partly explain why leaf TNC concentrations (but not leaf N) were so strongly correlated with R measured at 25°C. Given that R showed similar patterns of thermal acclimation at the canopy-scale in the same trees (Drake *et al.*, 2016), our results highlight the potentially important role of leaf TNC in regulating thermal acclimation of R across scales.

We found only a weak positive association between $A_{\text{max}}^{25}$ and leaf N after accounting for variation in leaf mass associated with TNC (i.e. TNC-free $N_{\text{area}}$). In fact, seasonal changes in TNC had a large impact on $N_{\text{area}}$ via TNC effects on leaf mass; TNC accounted for a large percentage of leaf dry mass (24% on average). Other studies in eucalypts have found similarly high concentrations of TNC (Hume *et al.*, 1996; Quentin *et al.*, 2011). It is possible that the activation state of Rubisco (Cheng & Fuchigami, 2000) or N partitioning to different pools of photosynthetic proteins are more important predictors of photosynthetic capacity than total leaf N (Poorter & Evans, 1998; Takashima *et al.*, 2004; Onoda *et al.*, 2005).

Although seasonal changes in photosynthetic capacity were not linked with changes in leaf N, they were positively associated with seasonal changes in leaf TNC concentrations. In some species and elevated CO$_2$ studies, in particular, carbohydrate accumulation leads to downregulation of photosynthetic capacity (Goldschmit & Huber, 1992; Paul & Foyer, 2001; Medlyn *et al.*, 2002; Ainsworth *et al.*, 2004), which would result in a negative relationship between leaf TNC concentrations and photosynthetic capacity. However, the positive relationship between leaf TNC concentrations and $A_{\text{max}}^{25}$ we observed suggests that higher photosynthetic capacity was associated with an increase in leaf carbohydrates.

The negative correlations between leaf TNC relative to stem growth and leaf $R/A$ ratios (Fig. 7) suggest that source–sink imbalances influenced the temporal patterns of leaf TNC and thus acclimation of leaf A and R. During cold conditions, leaf A was high and $R/A$ ratios were low, whereas the C sink to growth was also low. This was associated with an accumulation of leaf TNC, which appears to have influenced the acclimation of leaf R and $A_{\text{max}}$. During warm conditions, by contrast, leaf A was reduced and $R/A$ ratios increased, whereas the C sink for growth also increased. This was associated with a draw-down of leaf TNC, again with implications for the acclimation of leaf R and $A_{\text{max}}$. These findings highlight the potentially important role of source–sink dynamics in driving physiological acclimation to temperature. Even so, these relationships remain correlative, and information about the age and location of C pools fuelling tree growth is required to fully understand source–sink dynamics in these trees (Keel *et al.*, 2007; Richardson *et al.*, 2013; Palacio *et al.*, 2014).

The physiological response of forest trees to changes in temperature represents one of the largest uncertainties in predictions of terrestrial ecosystem responses to climate change, and climate–C cycle feedbacks. In our study, *E. tereticornis* demonstrated acclimation of leaf A and R to seasonal temperature changes that was consistent under current and + 3°C warmer climate. Our results identified clear linkages between acclimation of A and R and seasonal changes in leaf TNC concentrations that were likely influenced by seasonal source–sink imbalances, and potentially support a substrate-based model of plant acclimation to temperature (Azcón-Bíeto *et al.*, 1983; Dewar *et al.*, 1999; Atkin & Tjoelker, 2003). Importantly, our results indicate that observations of thermal acclimation to prevailing seasonal temperature changes may be used to infer photosynthetic and respiratory responses of trees to climate warming.

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**Author contributions**

M.J.A. contributed to the study design, collected data, and led data processing, interpretation and writing. J.E.D. made substantial contributions to the study design, data collection and interpretation, and writing. C.C. contributed to data collection and writing. A.V. contributed substantially to sample processing, carbohydrate analyses and writing. O.G. and D.T.T. assisted with data interpretation and writing. P.B.R. made conceptual contributions, and assisted with data interpretation and writing. M.G.T. provided project leadership, contributed to the study design, collected data, made conceptual contributions, and contributed substantially to data interpretation and writing.

**References**


**Supporting Information**

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Mean daily air temperature (*Tₐₘ*) and vapour pressure deficit (VPD) over time for ambient and warmed (ambient *Tₐₘ* +3°C) temperature treatments.

**Fig. S2** The relationship between light- and CO₂-saturated net photosynthesis measured at 25°C (*A*ₘₐₓ) derived from photosynthetic CO₂-response (*A*–*C*ₐ) curves, and the maximum carboxylation rate of Rubisco (*V*ₐₘₐₓ), and the maximum rate of electron transport required to regenerate RuBP (*J*ₐₘₐₓ) measured at 25°C derived from the same *A*–*C*ₐ curves measured in *Eucalyptus tereticornis* trees grown under ambient and warmed (+3°C) temperatures.

**Fig. S3** Short-term instantaneous temperature response curves of light- and CO₂-saturated (reference [CO₂] = 1800 μmol mol⁻¹) net photosynthesis (*A*ₘₐₓ) of individual *Eucalyptus tereticornis*
leaves measured at a range of leaf temperatures ($T_{\text{leaf}}$) in both temperature treatments (ambient, warmed) in spring, summer and autumn.

**Fig. S4** Rates of mass-based dark respiration ($R_{\text{mass}}$) measured at a fixed measurement temperature ($25^\circ\text{C}$; $R_{\text{mass}}^{25}$) in *Eucalyptus tereticornis* in relation to measurement date and prevailing mean daily (3-d mean) air temperature ($T_{\text{air}}$), as well as the relationship between in situ $R_{\text{mass}}$ measured at prevailing conditions in relation to measurement date and mean measurement leaf temperature ($T_{\text{leaf}}$).

**Fig. S5** The relationship between light- and CO$_2$-saturated leaf net photosynthesis measured at $25^\circ\text{C}$ ($A_{\text{max}}^{25}$) and light-saturated net photosynthesis ([CO$_2$] = 400 µmol mol$^{-1}$) measured at the mean measurement leaf temperature ($A_{\text{net}}$).

**Fig. S6** Mean (± SE) leaf mass area (LMA) and leaf N per unit area ($N_{\text{area}}$) over time and in relation to prevailing mean daily (3-d mean) air temperature ($T_{\text{air}}$), and mean (± SE) TNC-free LMA and TNC-free $N_{\text{area}}$ over time and in relation to mean daily $T_{\text{air}}$.

**Table S1** Correlation coefficients describing the relationship between 3-, 5-, 10-, 15- and 30-d mean daily minimum, mean daily, and mean daily maximum air temperatures and photosynthetic and respiratory capacity measured at a set temperature ($25^\circ\text{C}$) in *Eucalyptus tereticornis*.

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