Strong thermal acclimation of photosynthesis in tropical and temperate wet-forest tree species: the importance of altered Rubisco content

ANDREW P. SCAFARO1,*, SHUANG XIANG2,3,*, BENEDICT M. LONG3,4, NUR H. A. BAHAR1, LASANTHA K. WEERASINGHE5, DANIELLE CREEK6, JOHN R. EVANS3,4, PETER B. REICH6,7 and OWEN K. ATKIN1

1ARC Centre of Excellence in Plant Energy Biology, Research School of Biology, The Australian National University, Building 134, Canberra, ACT 2601, Australia, 2Chengdu Institute of Biology, Chinese Academy of Sciences, No. 9, Section 4, Rennmin South Road, Chengdu Sichuan 610041, China, 3Division of Plant Sciences, Research School of Biology, The Australian National University, Building 46, Canberra, ACT 2601, Australia, 4ARC Centre of Excellence for Translational Photosynthesis, Research School of Biology, The Australian National University, Building 134, Canberra, ACT 2601, Australia, 5Faculty of Agriculture, University of Peradeniya, 20400 Peradeniya, Sri Lanka, 6Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia, 7Department of Forest Resources, University of Minnesota, 1540 Cleveland Avenue North, St. Paul, MN 55108, USA

Abstract

Understanding of the extent of acclimation of light-saturated net photosynthesis (An) to temperature (T), and associated underlying mechanisms, remains limited. This is a key knowledge gap given the importance of thermal acclimation for plant functioning, both under current and future higher temperatures, limiting the accuracy and realism of Earth system model (ESM) predictions. Given this, we analysed and modelled T-dependent changes in photosynthetic capacity in 10 wet-forest tree species: six from temperate forests and four from tropical forests. Temperate and tropical species were each acclimated to three daytime growth temperatures (Tgrowth): temperate – 15, 20 and 25 °C; tropical – 25, 30 and 35 °C. CO2 response curves of An were used to model maximal rates of RuBP (ribulose-1,5-bisphosphate) carboxylation (Vcmax) and electron transport (Jmax) at each treatment’s respective Tgrowth and at a common measurement T (25 °C). SDS-PAGE gels were used to determine abundance of the CO2-fixing enzyme, Rubisco. Leaf chlorophyll, nitrogen (N) and mass per unit leaf area (LMA) were also determined. For all species and Tgrowth, An at current atmospheric CO2 partial pressure was Rubisco-limited. Across all species, LMA decreased with increasing Tgrowth. Similarly, area-based rates of Vcmax at a measurement T of 25 °C (Vcmax25) linearly declined with increasing Tgrowth linked to a concomitant decline in total leaf protein per unit leaf area and Rubisco as a percentage of leaf N. The decline in Rubisco constrained Vcmax and An for leaves developed at higher Tgrowth and resulted in poor predictions of photosynthesis by currently widely used models that do not account for Tgrowth-mediated changes in Rubisco abundance that underpin the thermal acclimation response of photosynthesis in wet-forest tree species. A new model is proposed that accounts for the effect of Tgrowth-mediated declines in Vcmax25 on An, complementing current photosynthetic thermal acclimation models that do not account for T sensitivity of Vcmax25.

Keywords: climate modelling, earth system models, photosynthesis, photosynthesis modelling, Rubisco content, temperature, thermal acclimation, tropical trees, Vcmax

Received 26 June 2016 and accepted 16 October 2016

Introduction

Earth system models (ESMs) are used to predict current and future carbon fluxes between terrestrial ecosystems and the atmosphere (Arora et al., 2013; Dufresne et al., 2013). ESM land surface components rely heavily on understanding how environmental factors such as temperature (T) affect leaf-level carbon assimilation (Canadell et al., 2007; Beer et al., 2010). Given that global warming is leading to higher average daily temperatures in many biomes, how light-saturated rates of net photosynthetic CO2 uptake (An) will respond to sustained changes in daytime growth temperature (Tgrowth) is a critical consideration for ESM predictions. It has long been known that An often acclimates to sustained increases in Tgrowth (Berry & Bjorkman, 1980), via physiological, structural or biochemical adjustments that can contribute to increased optimum temperatures (Topp) for An and/or altered photosynthetic capacity, when plants experience sustained higher Tgrowth.
(Lambers et al., 1998; Sage & Kubien, 2007; Smith & Dukes, 2013). Acclimation can result in rates of \( A_n \) being similar in cold and warm grown plants, when measured at prevailing \( T_{growth} \) of each treatment (i.e. homoeostasis). The degree of photosynthetic acclimation to temperature appears to be species and plant functional type (PFT) specific. For example, in a recent analysis of 70 studies (Way & Yamori, 2014), 76 of 150 species displayed increased \( T_{opt} \) and/or homoeostasis of \( A_n \) when they had acclimated to warmer \( T_{growth} \) conditions (termed constructive adjustment). In the remaining species, high \( T_{growth} \) was not associated with increased \( T_{opt} \) of \( A_n \) – in such cases, \( A_n \) at any given measuring \( T \) declines (termed detractive adjustment) in plants grown at higher \( T_{growth} \) (Way & Yamori, 2014). Interestingly, evergreen trees displayed constructive adjustments in only 36% of cases, less than C3 herbs, C4 plants or deciduous trees (Way & Yamori, 2014).

Notably, there are limited studies on photosynthesis acclimation for wet-forest tree species (Cunningham & Read, 2002, 2003; Cheesman & Winter, 2013). This is a problem for ESMs, as wet-forest trees – particularly those growing in the tropics – represent a major component of the global carbon cycle. Globally, biomass accumulation in tropical forests is estimated to have been 2369 Tg C yr\(^{-1} \) in recent years, compared with 574 Tg C yr\(^{-1} \) for boreal and temperate forests combined (Pan et al., 2011), highlighting the urgent need to understand how changes in \( T_{growth} \) affect photosynthesis of tropical species, including those growing in wet-forest ecosystems. Given the limited seasonal variations in \( T_{growth} \) in tropical regions (relative to the large seasonal variations in many temperate regions), some have speculated that wet-forest species from tropical equatorial regions may be less capable of acclimating than their temperate wet-forest counterparts (Cunningham & Read, 2003).

One powerful tool for understanding the mechanisms underpinning thermal acclimation of \( A_n \) is the widely used C3 photosynthesis model of Farquhar et al. (1980). By measuring the response of \( A_n \) to changes in intercellular \( CO_2 \) partial pressure, the model allows for determination of when photosynthesis is ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) limited, or limited by the regeneration of the Rubisco substrate, ribulose-1,5-bisphosphate, termed \( A_n \) and \( A_t \) limited, respectively. The maximum velocity of \( CO_2 \) carboxylation (\( V_{cmax} \)) and the maximum photosynthetic electron transport capacity, driving RuBP regeneration, (\( I_{max} \)), are two important photosynthetic variables that can be calculated from the model. Triose phosphate utilization of photosynthetic product can be a third limitation on \( A_n \) capacity, but usually occurs at higher than physiologically relevant \( CO_2 \) partial pressures (Sharkey, 1985; Sharkey et al., 2007), although it can limit photosynthesis at low temperatures (Sage & Kubien, 2007) and when phosphate content at the site of photosynthesis is low (Ellsworth et al., 2015). Although at current atmospheric \( CO_2 \) partial pressures, light-saturated rates of \( A_n \) are predominantly \( A_n \), \( A_t \), or colimited by both (Sage & Kubien, 2007), \( A_n \) is often \( A_n \) limited, especially at \( T_{opt} \), particularly in trees (Hikosaka et al., 2006; Sage et al., 2008). Hence, \( V_{cmax} \) is often the critical component in determining photosynthetic capacity. \( V_{cmax} \) is determined by enzyme kinetics of Rubisco. Rubisco enzymatic activity at saturating substrate levels increases exponentially with \( T \) (von Caemmerer, 2000). Consequently, \( V_{cmax} \) exponentially increases with \( T \) until excess heat reduces the enzymatic capacity of Rubisco in vivo (Medlyn et al., 2002a; Hikosaka et al., 2006; Kattge & Knorr, 2007). The in vivo impairment of Rubisco activity at high \( T \) is unlikely to be due to impairment of Rubisco itself, which is heat stable to 45 °C and above (Crafts-Brandner & Salvucci, 2000; Sage, 2002; Yamori et al., 2006). Rather, inactive Rubisco at high \( T \) is often attributed to Rubisco activase (RCA), the heat-labile chaperone of Rubisco, responsible for keeping Rubisco active (Portis, 2003). It is well known that RCA is susceptible to high \( T \) and this susceptibility leads to a decline in carboxylation activity of Rubisco (Crafts-Brandner & Salvucci, 2000; Salvucci & Crafts-Brandner, 2004a; Sage et al., 2008). Importantly, synthesis of a heat-stable RCA is thought to be one of the main drivers of the acclimation process to increased \( T_{growth} \) (Sage & Kubien, 2007).

In situations where higher \( T_{growth} \) is not associated with a change in \( V_{cmax} \) at a common measurement \( T \) – usually taken as 25 °C (\( V_{cmax}^{25} \)) – then \( A_n \) is expected to increase with increasing \( T_{growth} \) given the exponential \( T \) response of \( V_{cmax} \) at least until RCA-mediated limitations start to play a role. Indeed, there are reports that indicate that \( V_{cmax}^{25} \) may not correlate strongly with \( T_{growth} \) (Kattge & Knorr, 2007; Way & Oren, 2010). \( V_{cmax}^{25} \) values do, however, differ among PFTs (Kattge et al., 2009; Ali et al., 2015). Moreover, there are reports that \( T_{growth} \)-dependent shifts in \( V_{cmax}^{25} \) occur in some species. For example, the arid shrub Nerium oleander L. (Badger et al., 1982), cool climate herbaceous spinach (Spinacia oleracea) (Yamori et al., 2005) and maritime pine, Pinus pinaster Ait. (Medlyn et al., 2002b), all show declines in \( V_{cmax}^{25} \) when growing at warmer \( T \). In many cases, thermal acclimation also changes N partitioning between photosynthetic components and the ratio of \( I_{max} \) to \( V_{cmax} \) (when measured at 25 °C) which can lead to shifts in the \( A_n \) limitation dependent on \( T_{growth} \) (Hikosaka, 1997; Hikosaka et al., 1999; Onoda et al., 2005; Yamori et al., 2005, 2010; Kromdijk & Long, 2016). Given the possibility of common \( T_{growth} \)
adjustments to N partitioning and $V_{\text{cmax}}$\textsuperscript{25}, it is surprising that currently ESMs estimate $V_{\text{cmax}}$ based on a simple assumed fixed relationship between $V_{\text{cmax}}$ and N (Rogers, 2013).

The aim of our study was to determine how a wide range of $T_{\text{growth}}$ values (15–35 °C) affect rates of light-saturated $A_n$ and associated photosynthetic resource partitioning and capacity in 10 wet-forest tree species. We include both temperate (six) and tropical (four) species, beneficial in that modelling of wet-forest species photosynthesis in relation to temperature is underrepresented in the literature. We hypothesized: (1) that $T_{\text{growth}}$ acclimation over a wide $T$-range will include adjustments to photosynthetic capacity; and (2), that underpinning the acclimation to higher $T_{\text{growth}}$ are decreases in nitrogen partitioning to photosynthetic enzymes and resultant decreases in photosynthetic capacity. Species were grown 5 °C above and below their endemic mean maximum temperature by growing temperate species from 15 to 25 °C and tropical species from 25 to 35 °C. Therefore, comparison of acclimation properties between temperate and tropical individuals should not be confounded by one biome being exposed to temperatures further from that expected under in situ conditions. This is reasonable considering long-term (weeks to months) growth of a temperate species at 35 °C is as unrealistic of natural conditions as long-term growth of tropical species at 15 °C.

### Materials and methods

#### Plant material and growth treatments

Six broad-leaf, evergreen, wet-forest temperate species from the southeast region of Australia (*Anoperus glandulosus, Atherosperma moschatum, Eucryphia lucida, Eucalyptus regnans, Hedycarya angustifolia, Tasman尼亚 lanceolata*), and four broad-leaf, evergreen, wet-forest tropical species from the southeast region of Australia (*Castanopsisrum australis, Cryptocarya mack-innoniana, Synima cordioryyn, Syzygium sayeri*) were purchased from nurseries located in close proximity to each species’ provenance (Table S1). The mean maximum temperature of the warmest month is 20.1 ± 0.8 °C for temperate and 30.0 ± 0.4 °C for tropical sites. Annual precipitation means for the two wet-forest biomes are similar and between 1700 and 1900 mm yr\textsuperscript{-1}. Plants were 30–45 cm in height upon arrival at the Australian National University (Canberra, ACT). Plants were re-potted into 2.8 L volume, free-draining pots, containing an organic potting mix, enriched with Osmocote\textsuperscript{®}, OSEX34 EXACT slow-release fertilizer with an N/P/K ratio of 16 : 3.9 : 10 (Scotts Australia, Bella Vista, NSW, Australia). Fertilizer was applied at a rate of 3 kg m\textsuperscript{-3} of potting mix, and re-applied after 2–3 months, ensuring luxury supply of nutrients throughout the experiment. Plants were watered daily to field capacity.

Re-potted plants were placed in temperature-controlled growth chambers (Thermoline, Wetherhill Park, Australia) located in the Research School of Biology ‘Control Environment Growth Facility’ at the ANU, Canberra, Australia. Plants were exposed to a constant photosynthetically active irradiance of 700–800 μmol photons m\textsuperscript{-2} s\textsuperscript{-1} and 70–80% relative humidity at [CO\textsubscript{2}] of 400 μmol mol\textsuperscript{-1} (38.4 Pa, considering a mean atmospheric pressure of 96 kPa at the site of measurements) inside the growth chambers during the duration of the experiment. A 12 : 12-h dark : light photoperiod was maintained using 1000 Watt metal halide lamps (Multi-Vapor\textsuperscript{®}, GE Lighting, Derrimut, Australia).

Before exposing plants to different $T_{\text{growth}}$, plants were grown in a $T$-controlled growth chamber for 1.5 months at 30/25 °C (day/night) for tropical species and at 20/15 °C for temperate species, approximating $T_{\text{growth}}$ experienced by the selected tropical and temperate wet-forest species during the summer months (Xiang et al., 2013). The establishment period also enabled the seedlings to recover from transportation and transplanting shock. Plants were then either kept in the initial establishment $T_{\text{growth}}$ or moved to matched growth cabinets (with relative humidity, CO\textsubscript{2} and growth irradiances identical to the initial condition) where the day/night growth $T$ was 5 °C cooler or 5 °C warmer than the initial establishment condition. Consequently, tropical plants were exposed to day/night temperatures of 25/20 °C, 30/25 °C and 35/30 °C, and temperate plants were exposed to day/night regimes of 15/10 °C, 20/15 °C and 25/20 °C.

All experiments commenced 55 days after transferring to temperature treatments, on fully expanded leaves that developed during each temperature treatment. Temperate and tropical species were measured at $T_{\text{growth}}$, and at a common temperature of 25 °C by moving plants to the 25 °C growth cabinet prior to measurements. For all experiments, 3–5 biological replicates were sampled for each species, leading to a total of 114 individual analyses.

#### Gas-exchange measurements

All gas-exchange measurements were made using a Li-6400XT infrared gas-exchange analyser (LICOR, Lincoln, NE, USA). Intercellular CO\textsubscript{2} (C\textsubscript{i}) dependence of net assimilation ($A_n$) was determined by generating $A_n$-C\textsubscript{i} curves by adjusting Li-6400XT reference chamber CO\textsubscript{2} partial pressures, in the following chronological order: 38.4, 28.8, 19.2, 14.4, 12.0, 9.6, 7.2, 4.8, 2.4, 38.4, 57.6, 86.4, 120, 144, 163.2 and 192 Pa. Ten measurements were logged for each set point after photosynthesis stabilization, and all measurements included when generating $A_n$-C\textsubscript{i} curves. Photosynthetically active radiation (PAR) was set to 1800 μmol photons m\textsuperscript{-2} s\textsuperscript{-1} and a flow rate of 500 μmol s\textsuperscript{-1} for all $A_n$-C\textsubscript{i} measurements. At the completion of the final CO\textsubscript{2} measurement, reference CO\textsubscript{2} was returned to 38.4 Pa and the leaf within the cuvette was dark adapted for 30 min before a final 10 measurements were logged for capturing dark respiration rates ($R_{\text{dark}}$). The Licor ‘block’ $T$ was set to the greenhouse temperature, and the mean leaf $T$ for every $A_n$-C\textsubscript{i} curve was within ±1.3 °C of ambient air. The vapour pressure deficit based on leaf temperature ranged
from 1 to 3.2 kPa across all measurement temperatures and relative humidity ranged from 46% to 66%. Subsequent to gas-exchange measurements, gasket CO₂ diffusion was corrected for, as previously described (Bruhn et al., 2002).

**Photosynthesis modelling**

$A_n$-C₃ were converted to $A_n$-C₄ curves, with partial pressure (Pa) of CO₂ at the site of the chlorophyll ($C_m$), calculated from

$$C_m = C_1 - \frac{A_n}{g_m},$$

(1)

where $g_m$ (µmol m⁻² s⁻¹ Pa⁻¹) is the mesophyll conductance. $g_m$ at 25°C was set to 3.1 µmol m⁻² s⁻¹ Pa⁻¹ for all species, calculated from the average of two temperate tree species (Eucalyptus pauciflora and Quercus engelmannii), and one tropical species (Lophodermion confertus), measured using carbon isotope discrimination (von Caemmerer & Evans, 2015). $g_m$ was adjusted to measurement temperature using the equation:

$$g_m = \frac{1}{g_m} + \frac{T}{g_m},$$

(2)

where $g_m$ is conductance through the liquid phase (0.36 µmol s⁻¹ at 25°C) and $g_{mem}$ is conductance through the membrane phase (0.0484 µmol s⁻¹ at 25°C), and the energy of activation (61.7 kJ mol⁻¹) determined the temperature response of $g_{mem}$ was taken as the average values of the three aforementioned species [refer to von Caemmerer & Evans (2015) for comprehensive details].

Photosynthesis was modelled using the C₃ photosynthesis model of Farquhar et al. (1980), assuming either Rubisco ($A_r$) or RuBP regeneration ($A_b$) limitation. $A_r$ limited photosynthesis was modelled using the equation

$$A_r = \frac{V_{cmax}(C_1 - \Gamma^*)}{C_1 + K_c(1 + \frac{c}{k_c})} - R_{light},$$

(3)

where $V_{cmax}$ (µmol m⁻² s⁻¹) is the maximum rate of RuBP carboxylation, $K_c$ (27.24 Pa) and $K_o$ (16.58 kPa) are the Rubisco Michaelis constants for CO₂ and O₂, respectively, $O$ (21 kPa) is the O₂ partial pressure, $R_{light}$ is the light respiration, and $\Gamma^*$ is the CO₂ compensation point in the absence of respiration (3.74 Pa). $K_c$ and $k_c$ are the scaling constants (c) of 13.49, 13.38 and 14.68, respectively, and the energy of activation ($E_a$) of 24.46, 10.99 and 23.72, respectively, was taken from tobacco (Bernacchi et al. 2002). $V_{cmax}$ and $R_{light}$ are initially set to $y$-axis maximum and the value of $y$ at x-axis minimum, respectively, and were solved by iteration, using a nonlinear least-squares fit, using the graph and statistical software GraphPad Prism 5.0d (GraphPad Prism Software, Inc., San Diego, CA, USA). Through visual inspection, the $A_r$ curve was fit only to $A_r$ measurements below a $C_c$ value which corresponded with Rubisco limitation. Where the transition point was ambiguous, reducing the sum of squares of the modelled curve refined the points fitted. RuBP-limited photosynthesis ($A_b$) was modelled using the equation:

$$A_b = \frac{J_{max}(C_1 - \Gamma^*)}{4C_1 + H_1} - R_{light},$$

(4)

where $J_{max}$ is the maximum rate of chloroplast electron transport. For $A_r$, $R_{light}$ was taken as the value solved using $A_r$ modelling. $J_{max}$ was solved by iteration, for $A_r$ measurements above a $C_c$ value, as explained above.

**Temperature response functions**

The temperature response of $V_{cmax}$ and $J_{max}$ was modelled in two commonly reported ways, differing in that one is generated from a single $T_{growth}$, while the other incorporates thermal acclimation adjustments in $V_{cmax}$ and $J_{max}$ to sustained differences in $T_{growth}$. The first method, not incorporating acclimation, was using the standard Arrhenius function:

$$V_{cmax} = V_{cmax}^{ref} \exp \left( \frac{-D}{T} \right).$$

(5)

with the parameter being either $V_{cmax}$ or $J_{max}$ measured at 25°C. $c$ is the scaling constant, and $E_a$ is the energy of activation (both presented in kJ mol⁻¹). For $V_{cmax}$ and $E_a$ were set to 26.36 and 65.33, respectively, and for $J_{max}$ and $E_a$ were set to 17.71 and 43.9, respectively, obtained from in vivo measurements of tobacco grown at 25°C (Bernacchi et al., 2002). Alternatively, for $V_{cmax}$ and $E_a$ were solved by iteration in the same manner as $A_r$ and $A_b$ mentioned above. $R$ is the molar gas constant (8.314 J mol⁻¹ K⁻¹), and $T_1$ is the temperature of the leaf in Kelvin (K).

The second method used a peaked model, with refinement to account for acclimation driven changes in the $T$-optimum of $V_{cmax}$ or $J_{max}$ by Kattge & Knorr (2007), with $V_{cmax}$ and $J_{max}$ calculated as:

$$V_{cmax} = V_{cmax}^{ref} \exp \left( \frac{-D}{T - T_{growth}} \right).$$

(6)

and,

$$J_{max} = \left[ a_{T_{opt}}(T) + b_{T_{opt}}(T) \right] V_{cmax}^{ref} \exp \left( \frac{-D}{T - T_{growth}} \right).$$

(7)

where $V_{cmax}^{ref}$ is the base rate of $V_{cmax}$ at a measured temperature of 25°C. $T_1$ is the leaf temperature and $T_{opt}$ is the reference temperature (i.e. 25°C), both expressed in Kelvin. The enthalpy ($H_a$) was set at 71.5 kJ mol⁻¹ for $V_{cmax}$ and 50 kJ mol⁻¹ for $J_{max}$. $H_d$ is the deactivation enthalpy and was set at 200 kJ mol⁻¹ for both $V_{cmax}$ and $J_{max}$. The term ($a_{T_{opt}}+b_{T_{opt}} \times T_{growth}$) is the entropy factor ($\Delta S$) adjusted for the growth temperature ($T_{growth}$ in °C), by taking into account the intercept (a) and slope (b) of a linear regression analysis between $\Delta S$ and $T_{growth}$ for $V_{cmax}$ (0.6684 kJ mol⁻¹ and −1.07 kJ mol⁻¹ °C⁻¹ for a and b, respectively) or $J_{max}$ (0.6597 kJ mol⁻¹ and −0.75 kJ mol⁻¹ °C⁻¹ for a and b).
respectively. For the nonacclimated model of \( V_{\text{cmax}} \) and \( J_{\text{max}} \), \( \Delta S \) was set at 0.649 kJ mol\(^{-1}\) and 0.646 kJ mol\(^{-1}\) °C\(^{-1}\), respectively. All parameters, except for the term described below, were taken from Kattge & Knorr (2007).

To adjust \( J_{\text{max}} \) to the base rate of \( V_{\text{cmax}} \), the intercept and slope of the fraction of \( J_{\text{max}} \) to \( V_{\text{cmax}} \) at 25 °C was accounted for through the term \( a_{\text{J,V}} * b_{\text{J,V}} * T_{\text{growth}} \) with the intercept and slope set to 2.9 and -0.032, respectively, derived from the \( J_{\text{max}} \) to \( V_{\text{cmax}} \) values at 25 °C (\( J_{\text{max}} / V_{\text{cmax}} \)), obtained in this study (Table 1), rather than the \( J_{\text{max}} / V_{\text{cmax}} \) presented by Kattge & Knorr (2007).

**Leaf mass, area, nitrogen, chlorophyll and Rubisco quantification**

Leaves were collected following gas-exchange measurements. Initially, the fresh mass of leaf material used in the gas-exchange measurements was determined (Mettler-Toledo Ltd, Port Melbourne, Vic, Australia); thereafter, leaf area was measured using a LI-3100 leaf area meter (Li-COR, Inc. Lincoln, NE, USA). Gas-exchange leaves were oven dried at 70 °C for >48-h, weighed and leaf dry mass per unit leaf area (LMA) calculated. Total N of dried leaves was calculated using the Kjeldahl acid digest method (Allen et al., 1974). Additional measurements (i.e. chlorophyll) and protein abundance) were also made on leaf discs taken from the closest, adjacent fully expanded leaf to that used in the gas-exchange measurements. Chlorophyll content of two 0.77 cm² snap-frozen leaf discs was extracted by grinding in a mortar and pestle, in acetone with 2.5 mM MgCO₃. The supernatant absorbance was read on a UV–VIS spectrometer (Lambda 25; Perkin Elmer, Shelton, CT, USA). Rubisco content was measured by homogenizing leaf discs into powder using a Qiagen Tissue-Lyzer 48 (Qiagen, Venlo, the Netherlands). Due to high phenolic content and rapid oxidation of samples during extraction, the application required a specific extraction procedure in order to allow the determination of Rubisco content. Protein was extracted from tissue powder using extraction buffer, following the method of Gaspar et al. (1997), as adapted by Bahar et al. (2016), with all procedures undertaken on ice. 4× SDS-PAGE sample buffer (Invitrogen, Carlsbad, CA, USA) was added to soluble protein and loaded in equal volumes on 4–12% NuPage Bis-Tris polyacrylamide gels (Invitrogen). Rubisco purified from *N. tabacum* (tobacco) was loaded onto gels in varying concentrations to generate a standard curve of Rubisco large subunit (LSU) quantities. The quantity of tobacco Rubisco used as a standard was predetermined using radiolabelled \(^{14}C\)carboxypentitol-P₂ (Ruuska et al., 1998). Gels were stained with GelCode Blue stain reagent (ThermoFisher Scientific, Scoresby, Vic, Australia), and image analysis of protein bands using a Versa-Doc (Bio-Rad, Hercules, CA, USA) provided quantification of Rubisco, by comparing purified standards with the corresponding Rubisco bands of each sample (Fig. S1).

**Statistical analysis**

Leaf trait means and standard deviations for each temperate and tropical species at each measured temperature are provided (Tables S2 and S3). We performed linear regression analysis of leaf traits as a function of \( T_{\text{growth}} \) for all species means. Temperate and tropical species data sets were treated separately and as one. If there was no difference in slopes or intercepts for the two data sets, as determined by an ANOVA of the slope and intercept means (Table 1), then linear regression analysis across the entire temperature range, irrespective of biome, was explored. For the temperature response curve analysis of \( V_{\text{cmax}} \) and \( J_{\text{max}} \), the non-linear goodness of fit (R²) is based on the mean value of all species combined at each \( T_{\text{growth}} \). All graphs, statistical analysis and model fits were achieved through the graph, statistical and curve fitting software GRAPHPAD PRISM 5.0d.

**Results**

**Growth temperature adjustments in leaf anatomical and chemical properties**

For many leaf traits, we found similar \( T_{\text{growth}} \) responses among species. For example, in most temperate and tropical wet-forest species, area-based Rubisco content and \( V_{\text{cmax}} \) decreased with increasing \( T_{\text{growth}} \) (Fig. S2). Given the general similarity in trait temperature responses in temperate and tropical species, we chose to show at each \( T_{\text{growth}} \) trait values averaged across all species within each biome group (e.g. Figs 1, 3 and 6), with regression analyses and ANOVAs (Table 1) being used to test whether there were significant differences in the slope and/or intercept of trait–\( T_{\text{growth}} \) relationships between the two biome groups; for these analyses, analyses were carried out using species mean values, but with the figures showing biome-group means. For most traits, there was no significant difference in the slopes and/or intercept means between the two biome groups (Table 1). Given this, one can fit a common regression across both biomes when plotting trait values against \( T_{\text{growth}} \) for those traits with significant \( T_{\text{growth}} \)-dependent changes in trait values. An example is the decline in area-based Rubisco content (Rubiscoₐ) with increasing \( T_{\text{growth}} \) (Fig. 1a). Although LMA also decreased with increasing \( T_{\text{growth}} \) (Fig. 1b), the fall in Rubiscoₐ was not due to this LMA decline alone, as there was an associated increase in N on a dry mass basis (\( N_m \)) with increasing \( T_{\text{growth}} \) (Fig. 1c), counteracting the reduction in LMA and leading to no significant decline in nitrogen on an area basis (\( N_a \); Fig. 1d). The decline in Rubiscoₐ was therefore not due to any \( T_{\text{growth}} \)-dependent change in \( N_a \) but rather a reduced allocation of N content to Rubisco, evident in Rubisco as a percentage of leaf N declining with increasing \( T_{\text{growth}} \) (Fig. 1e). Of note, no significant \( T_{\text{growth}} \)-dependent decrease in Rubisco on a dry mass basis was observed (Fig. 1f), as the decline in Rubisco as a percentage of N was counteracted by the increase in \( N_m \) with increasing \( T_{\text{growth}} \).
Table 1  Linear regression analysis and ANOVA of leaf trait (y-value) responses to daytime growth temperature (x-value), for temperate, tropical and both temperate and tropical species combined.

<table>
<thead>
<tr>
<th>Leaf trait</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
<th>$P$</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
<th>$P$</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMA</td>
<td>-2.09 ± 1.68</td>
<td>137 ± 34</td>
<td>0.09</td>
<td>0.230</td>
<td>-0.783 ± 0.704</td>
<td>92.4 ± 21.3</td>
<td>0.11</td>
<td>0.292</td>
<td>0.37 ± 0.549</td>
<td>0.63 ± 0.435</td>
<td>-2.20 ± 0.663</td>
<td>138 ± 16.5</td>
</tr>
<tr>
<td>$N_m$</td>
<td>0.185 ± 0.203</td>
<td>18.9 ± 4.2</td>
<td>0.05</td>
<td>0.377</td>
<td>0.42 ± 0.42</td>
<td>14.7 ± 12.7</td>
<td>0.09</td>
<td>0.339</td>
<td>0.32 ± 0.577</td>
<td>0.56 ± 0.461</td>
<td>0.397 ± 0.129</td>
<td>15.0 ± 3.2</td>
</tr>
<tr>
<td>$N_a$</td>
<td>-0.023 ± 0.032</td>
<td>2.56 ± 0.644</td>
<td>0.03</td>
<td>0.471</td>
<td>0.011 ± 0.022</td>
<td>1.51 ± 0.65</td>
<td>0.02</td>
<td>0.632</td>
<td>0.63 ± 0.435</td>
<td>0.38 ± 0.541</td>
<td>-0.020 ± 0.013</td>
<td>2.46 ± 0.33</td>
</tr>
<tr>
<td>Rubisco$_Ox_N$</td>
<td>-0.370 ± 0.160</td>
<td>14.4 ± 3.3</td>
<td>0.25</td>
<td>0.034</td>
<td>-0.196 ± 0.172</td>
<td>11.8 ± 5.2</td>
<td>0.12</td>
<td>0.280</td>
<td>0.52 ± 0.479</td>
<td>1.5 ± 0.238</td>
<td>-0.192 ± 0.076</td>
<td>11.2 ± 1.9</td>
</tr>
<tr>
<td>Rubisco$_O$</td>
<td>-0.763 ± 0.285</td>
<td>28.4 ± 5.8</td>
<td>0.31</td>
<td>0.016</td>
<td>-0.221 ± 0.261</td>
<td>16.2 ± 7.9</td>
<td>0.07</td>
<td>0.416</td>
<td>1.7 ± 0.197</td>
<td>0.48 ± 0.493</td>
<td>-0.438 ± 0.129</td>
<td>22.2 ± 3.2</td>
</tr>
<tr>
<td>Rubisco$_m$</td>
<td>-0.006 ± 0.03</td>
<td>0.27 ± 0.07</td>
<td>0.18</td>
<td>0.072</td>
<td>-0.003 ± 0.005</td>
<td>0.22 ± 0.16</td>
<td>0.02</td>
<td>0.628</td>
<td>0.43 ± 0.516</td>
<td>1.66 ± 0.210</td>
<td>-0.002 ± 0.002</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>$A_s$</td>
<td>-0.188 ± 0.195</td>
<td>13.6 ± 4.0</td>
<td>0.05</td>
<td>0.350</td>
<td>-0.101 ± 0.124</td>
<td>9.7 ± 3.8</td>
<td>0.06</td>
<td>0.433</td>
<td>0.11 ± 0.744</td>
<td>0.98 ± 0.331</td>
<td>-0.249 ± 0.081</td>
<td>14.5 ± 2.0</td>
</tr>
<tr>
<td>$A_m$</td>
<td>-0.272 ± 2.48</td>
<td>115 ± 51</td>
<td>&lt;0.00</td>
<td>0.914</td>
<td>-0.271 ± 2.60</td>
<td>10.9 ± 79</td>
<td>&lt;0.00</td>
<td>0.919</td>
<td>&lt;0.00</td>
<td>0.1</td>
<td>0.05 ± 0.819</td>
<td>-0.589 ± 1.12</td>
</tr>
<tr>
<td>$g_s$</td>
<td>-0.002 ± 0.005</td>
<td>0.18 ± 0.10</td>
<td>0.01</td>
<td>0.688</td>
<td>-0.002 ± 0.003</td>
<td>0.17 ± 0.08</td>
<td>0.07</td>
<td>0.413</td>
<td>-0.002 ± 0.977</td>
<td>0.21 ± 0.654</td>
<td>-0.003 ± 0.002</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>$C_i$</td>
<td>0.046 ± 0.11</td>
<td>25.4 ± 2.3</td>
<td>0.01</td>
<td>0.694</td>
<td>-0.120 ± 0.21</td>
<td>28.6 ± 6.4</td>
<td>0.03</td>
<td>0.502</td>
<td>0.57 ± 0.460</td>
<td>0.68 ± 0.418</td>
<td>-0.088 ± 0.068</td>
<td>27.9 ± 1.7</td>
</tr>
<tr>
<td>$C_A$</td>
<td>0.193 ± 0.13</td>
<td>19.0 ± 2.7</td>
<td>0.01</td>
<td>0.170</td>
<td>-0.0319 ± 0.22</td>
<td>24.2 ± 6.8</td>
<td>&lt;0.00</td>
<td>0.865</td>
<td>0.9 ± 0.352</td>
<td>0.24 ± 0.628</td>
<td>0.0550 ± 0.076</td>
<td>21.6 ± 1.9</td>
</tr>
<tr>
<td>$R_{light}$</td>
<td>-0.037 ± 0.047</td>
<td>3.6 ± 1.0</td>
<td>0.04</td>
<td>0.437</td>
<td>-0.020 ± 0.017</td>
<td>0.71 ± 0.51</td>
<td>0.12</td>
<td>0.273</td>
<td>0.91 ± 0.349</td>
<td>14 ± 0.01</td>
<td>-0.097 ± 0.022</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>$R_{dark}$</td>
<td>-0.013 ± 0.018</td>
<td>1.1 ± 0.4</td>
<td>0.03</td>
<td>0.466</td>
<td>0.016 ± 0.026</td>
<td>0.8 ± 0.8</td>
<td>0.04</td>
<td>0.548</td>
<td>0.01 ± 0.917</td>
<td>1.9 ± 0.182</td>
<td>-0.001 ± 0.009</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>$R_{dark}$/</td>
<td>-0.002 ± 0.001</td>
<td>0.075 ± 0.023</td>
<td>0.15</td>
<td>0.116</td>
<td>-0.002 ± 0.001</td>
<td>0.081 ± 0.017</td>
<td>0.49</td>
<td>0.011</td>
<td>&lt;0.000</td>
<td>0.059</td>
<td>0.64 ± 0.429</td>
<td>-0.0014 ± 0.0004</td>
</tr>
<tr>
<td>$V_{cmax}$</td>
<td>-1.13 ± 0.94</td>
<td>80 ± 19</td>
<td>0.08</td>
<td>0.250</td>
<td>-0.381 ± 0.843</td>
<td>48 ± 26</td>
<td>0.02</td>
<td>0.661</td>
<td>0.30 ± 0.586</td>
<td>2.2 ± 0.148</td>
<td>-1.57 ± 0.43</td>
<td>86 ± 11</td>
</tr>
<tr>
<td>$V_{cmax}$</td>
<td>-0.67 ± 0.74</td>
<td>44 ± 15</td>
<td>0.05</td>
<td>0.378</td>
<td>-0.332 ± 0.521</td>
<td>30 ± 16</td>
<td>0.04</td>
<td>0.539</td>
<td>0.11 ± 0.738</td>
<td>0.67 ± 0.419</td>
<td>-0.644 ± 0.278</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>$I_{max}$</td>
<td>-1.7 ± 2.1</td>
<td>165 ± 43</td>
<td>0.04</td>
<td>0.441</td>
<td>-1.2 ± 1.3</td>
<td>98 ± 39</td>
<td>0.08</td>
<td>0.373</td>
<td>0.03 ± 0.873</td>
<td>9.8 ± 0.04</td>
<td>-4.7 ± 0.9</td>
<td>218 ± 25</td>
</tr>
<tr>
<td>$I_{max}$/</td>
<td>0.021 ± 0.020</td>
<td>1.9 ± 0.4</td>
<td>0.07</td>
<td>0.268</td>
<td>-0.022 ± 0.014</td>
<td>2.5 ± 0.4</td>
<td>0.20</td>
<td>0.145</td>
<td>2.6 ± 0.118</td>
<td>12 ± 0.02</td>
<td>-0.032 ± 0.010</td>
<td>2.9 ± 0.2</td>
</tr>
</tbody>
</table>

*Indicates significant differences ($P < 0.05$) in the comparison and combined analysis.
Photosynthetic acclimation to growth temperature

The manner in which Rubisco (A_c) and RuBP regeneration (A_r) limitations regulate light-saturated rates of net photosynthesis (A_n) within increasing C_c is presented in Fig. 2. Results are shown separately for temperate and tropical trees (averaged across species), grown in the range of 15–35 °C. Individual species A_n–C_c responses are shown in Fig. S3. A_c models provided a high degree of accuracy (R^2 = 0.98 ± 0.02) in estimating the measured data. In general, temperate species became A_r limited at higher C_c than their tropical counterparts, although variation in transitions from A_c to A_r limitation is noted between species within each biome (Fig. S3). Even so, irrespective of T_grow or biome-origin, A_n at the current ambient CO_2 partial pressure (C_a) of 38.4 Pa, and both saturating light of 1800 μmol photons m^{-2} s^{-1} and without water limitations, was in all cases, Rubisco-limited (Fig. 2). Indeed, all biological replicates for all species were A_c limited at a C_a of 38.4 Pa (Fig. S3). In most cases, A_n did not become A_r limited until C_c partial pressure was >40 Pa, equivalent to a C_a of about 57.6 Pa, well above current C_a. A_c models did not fit the observations as well (r = 0.67 ± 0.18), perhaps reflecting J_{max} not being reached within the C_c range measured in all species (Fig. S3).

There was a significant negative linear relationship between light-saturated net assimilation (at current C_a of 38.4 Pa) on an area basis (A_a) and T_grow when temperate and tropical species were combined (Fig. 3a, Table 1). The adjustment of A_a was considerable, averaging ≈2.5 μmol m^{-2} s^{-1} for every 10 °C shift in T_grow. Area-based leaf stomatal conductance (g_s) followed a similar pattern as for A_a, with the g_s–T_grow relationship being significant at P = 0.11. Rates of net assimilation on a mass basis (A_m; again measured at T_grow) did not significantly vary with T_grow, indicating homoeostasis of mass-based carbon gain across the
Fig. 2 The modelled response of net assimilation ($A_n$) to changes in chloroplast CO$_2$ partial pressure ($C_c$) for six temperate tree species grown at 15, 20 and 25 °C (a), and four tropical tree species grown at 25, 30 and 35 °C (b). Solid lines indicate Rubisco limitation ($A_c$), and dashed lines indicate RuBP regeneration limitation ($A_r$). Open circles are $A_n$ values when ambient CO$_2$ was equal to 38.4 Pa. Each curve is the mean of 20, 20, 22, 19, 16 and 16 individually modelled $A_n$-$C_c$ data sets for temperate species at 15, 20 and 25 °C and tropical species at 25, 30 and 35 °C, respectively. Photosynthesis was modelled using the published Rubisco kinetic parameters of tobacco grown at 25 °C, as presented by Bernacchi et al. (2002).

Fig. 3 Net assimilation ($A_n$), stomatal conductance ($g_s$), internal CO$_2$ partial pressures ($C_i$ and $C_c$) and respiration ($R_{light}$ and $R_{dark}$) of temperate (blue circles) and tropical (magenta squares) tree species across the daytime growth temperature range of 15–35 °C ($T_{growth}$), at an ambient CO$_2$ partial pressure of 38.4 Pa. (a, b, c) Net assimilation, on an area ($A_a$) and dry mass basis ($A_m$), and stomatal conductance ($g_s$). (d) Intercellular CO$_2$ ($C_i$ – open symbols) and chloroplast CO$_2$ ($C_c$ – closed symbols). (e) $R_{light}$, extrapolated from $A_n$-$C_c$ modelling (open symbols), as well as $R_{dark}$ (closed symbols) from direct gas-exchange measurements. Note that rates of $R_{light}$ of the tropical species were near identical to those in darkness, and symbols are thus not visible. (f) $R_{dark}$ as a fraction of $V_{cmax}$. Solid black lines indicate significant ($P < 0.05$) linear regression of individual species means when temperate and tropical data sets were combined (refer to Table 1). Values are the mean ± standard error of the species means at each growth temperature.
20 °C range of \( T_{\text{growth}} \) values. Intercellular CO\(_2\) partial pressure (\( C_i \)) was stable over the entire temperature range, as was calculated chloroplast CO\(_2\) partial pressure (\( C_p \)) and the difference between the two never exceeded 4.3 Pa (Fig. 3d). Temperate species exhibited higher estimated \( R_{\text{light}} \) than tropical species (Fig. 3e; note that rates of \( R_{\text{light}} \) of the tropical species were near identical to those in darkness, and symbols are thus not visible), as seen by the significant difference in the \( y \)-intercept of regression analysis between the two biomes (Table 1). \( R_{\text{light}} \) did not show a significant linear decline with increased \( T_{\text{growth}} \) for each biome analysed individually (Fig. 3e, Table 1). Respiration in the dark (\( R_{\text{dark}} \)), measured directly from gas-exchange measurements in darkness at \( T_{\text{growth}} \) was stable across the entire \( T_{\text{growth}} \) range and values for temperate and tropical biomes were similar (Fig. 3e), indicating very strong thermal acclimation. Due to the temperature stability of respiration (i.e. homoeostasis) but not of \( V_{\text{cmax}} \), \( R_{\text{dark}} \) as a fraction of photosynthetic capacity (\( R_{\text{dark}}/V_{\text{cmax}} \)) significantly declined in response to increasing temperature (Fig. 3f).

\[ T_{\text{growth}} \] implications on the short-term temperature dependence of \( V_{\text{cmax}} \)

We assessed how acclimation to a wide range of \( T_{\text{growth}} \) impacted on the immediate temperature response of \( V_{\text{cmax}} \). To achieve this, the \( V_{\text{cmax}} \) of each species and \( T_{\text{growth}} \) was measured shortly after moving plants to a common 25 °C (\( V_{\text{cmax}25} \)). By plotting \( V_{\text{cmax}} \) measured at each respective \( T_{\text{growth}} \) as a fraction of \( V_{\text{cmax}} \) measured at 25 °C (\( V_{\text{cmax}25} \)), an immediate temperature response curve of \( V_{\text{cmax}} \) was generated (Fig. 4a). In doing so, we could assess the impact of short-term changes in measuring \( T \) on \( V_{\text{cmax}} \) values for cool and warm acclimated plants. The solid curves in Fig. 4a follow the standard Arrhenius function, with an exponential increase in \( V_{\text{cmax}} \) with rising temperature [red curve – derived from tobacco grown at 25 °C (Bernacchi et al., 2002); black curve – iteratively fit]. Interestingly, the Arrhenius functions provided excellent fits to the observed \( V_{\text{cmax}25} \) values of both temperate and tropical trees (\( R^2 = 0.97 \) for Bernacchi et al. (2002) or \( R^2 = 0.99 \) by iteration; Table 2), despite the modelled \( V_{\text{cmax}} \) temperature response curve of Bernacchi et al. (2002) being derived from tobacco, grown only at 25 °C, a powerful indication that Rubisco enzymatic properties, independent of activation state, do not significantly adjust to \( T_{\text{growth}} \).

Next, we used the peaked model of Kattge & Knorr (2007), which accounts for declines in \( V_{\text{cmax}} \) at above optimal \( T \). The peaked model parameterized to each measured \( T_{\text{growth}} \) fit closely with the corresponding

\[ V_{\text{cmax}}, \text{measurement, as well as all measurements below the set acclimation temperature. Thus, the peaked model, with the entropy factor (\( AS \)) adjusted to a \( T_{\text{growth}} \) of 35 °C, was a good fit (\( R^2 = 0.97 \)) to the entire range of \( V_{\text{cmax}} \) from 15 to 35 °C (Fig. 4a, Table 2). However, the peaked model without acclimation (i.e. no \( T_{\text{growth}} \) adjustments in \( AS \)) resulted in reduced accuracy (\( R^2 = 0.47 \)), supporting the need for an acclimation term in the peaked model. Taking both the Arrhenius

![Fig. 4](image-url)
and peaked model results together, these findings suggest that acclimation to a wide range of $T_{\text{growth}}$ values does not lead to short-term $T$-inhibition of $V_{\text{cmax}}$.

Using a similar approach as above to understand the drivers of acclimation of RuBP regeneration, we plotted $I_{\text{max}}$ as a fraction of each species’ $I_{\text{max}}$ measured at 25 °C ($I_{\text{max}}^{25}$), yielding values of $I_{\text{max}}^{g/25}$ (Fig. 4b). Unlike $V_{\text{cmax}}$ using the $T$ response curve of $I_{\text{max}}$ from tobacco grown at 25 °C (Bernacchi et al., 2002) did not fit the measurements of temperate and tropical trees in any meaningful way (Fig. 4b, Table 2). Using the peaked model (Kattge & Knorr, 2007) with an acclimation setting of 35 °C provided a reasonable fit ($R^2 = 0.74$), accounting for the diminishing increase in $I_{\text{max}}$ at high $T$. However, the fit was still poor, when compared to $V_{\text{cmax}}$ models, due to relatively high values of $I_{\text{max}}$ in temperate trees at 15 and 20 °C.

Rubisco content implications on the long-term temperature dependence of $V_{\text{cmax}}$

When $V_{\text{cmax}}$ was plotted at each prevailing $T_{\text{growth}}$, the long-term temperature dependence of $V_{\text{cmax}}$ is illustrated for temperate and tropical species (Fig. 5a). Both temperate and tropical species exhibited increases in $V_{\text{cmax}}$ with rising $T_{\text{growth}}$. However, when compared to the short-term temperature response function of $V_{\text{cmax}}$ reported by Bernacchi et al. (2002) (shown with solid curves), this long-term $V_{\text{cmax}}$ response to $T$ was above expectations at 15 and 20 °C, and below expectations at 35 °C (Fig. 5a). Further, when $V_{\text{cmax}}$ for each $T_{\text{growth}}$ was adjusted to account for the change in Rubisco content associated with $T_{\text{growth}}$ (Fig. 1a), the observed long-term $V_{\text{cmax}}$ values fit the expected short-term $V_{\text{cmax}}$ values to a much greater extent (Fig. 5b) with $R^2$ fits increasing from $-1.56$ (i.e. model fits worse than a horizontal line through the mean of $V_{\text{cmax}}$ values) to 0.86 for temperate and 0.61 to 0.95 for tropical species (Table 2). This was evident when comparing the deviation of observed relative to expected $V_{\text{cmax}}$. When not adjusting for changes in Rubisco content, $V_{\text{cmax}}$ varied by as much as 75% from that expected from the tobacco model (Fig. 5c). When changes in Rubisco were factored in, observed $V_{\text{cmax}}$ deviated by <25% from expected model values over the entire temperature range (Fig. 5d).

### Table 2 $V_{\text{cmax}}$ and $I_{\text{max}}$ temperature response curve analysis of growth acclimated temperate and tropical trees

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>Value at 25 °C</th>
<th>$c$</th>
<th>$E_a$ or $H_a$</th>
<th>$H_d$</th>
<th>$\Delta S$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arrhenius function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco grown at 25 °C</td>
<td>$V_{\text{cmax}}^{25}$</td>
<td>50.4</td>
<td>26.36</td>
<td>65.33</td>
<td>N/A</td>
<td>N/A</td>
<td>−1.56</td>
</tr>
<tr>
<td>Tobacco grown at 25 °C</td>
<td>$V_{\text{cmax}}^{25}$</td>
<td>36.6</td>
<td>26.36</td>
<td>65.33</td>
<td>N/A</td>
<td>N/A</td>
<td>0.610</td>
</tr>
<tr>
<td>Tobacco grown at 25 °C</td>
<td>$V_{\text{cmax}}^{25}$</td>
<td>50.4</td>
<td>26.36</td>
<td>65.33</td>
<td>N/A</td>
<td>N/A</td>
<td>0.855</td>
</tr>
<tr>
<td>Tobacco grown at 25 °C</td>
<td>$V_{\text{cmax}}^{25}$</td>
<td>36.6</td>
<td>26.36</td>
<td>65.33</td>
<td>N/A</td>
<td>N/A</td>
<td>0.953</td>
</tr>
<tr>
<td>Tobacco grown at 25 °C</td>
<td>$V_{\text{cmax}}^{25}$</td>
<td>1</td>
<td>26.36</td>
<td>65.33</td>
<td>N/A</td>
<td>N/A</td>
<td>0.968</td>
</tr>
<tr>
<td>Iteratively derived parameters</td>
<td>$V_{\text{cmax}}^{g/25}$</td>
<td>1</td>
<td>22.29 ± 1.4</td>
<td>55.01 ± 3.6</td>
<td>N/A</td>
<td>N/A</td>
<td>0.991</td>
</tr>
<tr>
<td>Tobacco grown at 25 °C</td>
<td>$I_{\text{max}}^{g/25}$</td>
<td>1</td>
<td>17.71</td>
<td>43.9</td>
<td>N/A</td>
<td>N/A</td>
<td>0.120</td>
</tr>
<tr>
<td><strong>Peaked model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 species without acclimation</td>
<td>$V_{\text{cmax}}^{g/25}$</td>
<td>1</td>
<td>N/A</td>
<td>71.5</td>
<td>200</td>
<td>0.649</td>
<td>0.473</td>
</tr>
<tr>
<td>36 species without acclimation</td>
<td>$V_{\text{cmax}}^{g/25}$</td>
<td>1</td>
<td>N/A</td>
<td>71.5</td>
<td>200</td>
<td>0.6309</td>
<td>0.965</td>
</tr>
<tr>
<td>36 species without acclimation</td>
<td>$V_{\text{cmax}}^{g/25}$</td>
<td>2.9</td>
<td>N/A</td>
<td>50</td>
<td>200</td>
<td>0.646</td>
<td>0.445</td>
</tr>
<tr>
<td>36 species without acclimation</td>
<td>$V_{\text{cmax}}^{g/25}$</td>
<td>2.9</td>
<td>N/A</td>
<td>50</td>
<td>200</td>
<td>0.6335</td>
<td>0.744</td>
</tr>
<tr>
<td><strong>Variable-base model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhenius function</td>
<td>$V_{\text{cmax}}$</td>
<td>51</td>
<td>22.29</td>
<td>55.01</td>
<td>N/A</td>
<td>N/A</td>
<td>0.932</td>
</tr>
<tr>
<td>Peaked model</td>
<td>$V_{\text{cmax}}$</td>
<td>46.8</td>
<td>N/A</td>
<td>71.5</td>
<td>200</td>
<td>Var#</td>
<td>0.924</td>
</tr>
</tbody>
</table>

The temperature response of $V_{\text{cmax}}$ and $I_{\text{max}}$ was modelled using either the Arrhenius function and parameters provided by Bernacchi et al. (2002), the peaked model with and without acclimation by Kattge & Knorr (2007) or the variable-base model proposed from this study. The scaling constant ($c$) and energy of activation ($E_a$) for the Arrhenius function, and the enthalpy ($H_d$), and deactivation enthalpy ($H_a$) in kJ mol$^{-1}$ for the peaked function, were provided, or calculated, from the corresponding published models, except where $c$ and $E_a$ were estimated by iteration using a least-squares nonlinear fit (with standard error values provided) and applied to the variable-base model. The entropy term ($\Delta S$) in kJ mol$^{-1}$ was calculated from the slope and intercept values of linear regression analysis, provided in Kattge & Knorr (2007). $V_{\text{cmax}}^{tr}$ refers to $V_{\text{cmax}}$ calculated for temperate species at growth temperature ($T_{\text{growth}}$); $V_{\text{cmax}}^{tr}$ refers to $V_{\text{cmax}}$ calculated for tropical species at $T_{\text{growth}}$; $V_{\text{cmax}}^{radj}$ and $V_{\text{cmax}}^{radj}$ refer to the $V_{\text{cmax}}$ of temperate and tropical trees with Rubisco adjusted to the content at the $T_{\text{growth}}$ of 25 °C. $V_{\text{cmax}}^{g/25}$ of $I_{\text{max}}^{g/25}$ refers to the parameter at $T_{\text{growth}}$ as a fraction of that measured at 25 °C. The nonlinear goodness of fit ($R^2$) was calculated from the combined species mean value at each temperature, and N/A refers to nonapplicable. Note that a negative $R^2$ means that the sum of squares for the model is greater than the sum of squares of the null hypothesis model, which is a horizontal line through the mean of all $y$-values. 

$^\text{#} N$ indicates a variable $\Delta S$ in response to $T_{\text{growth}}$. 

The Rubisco dependent improvement in model fits of $V_{cmax}$ plotted at each prevailing $T_{growth}$ (Fig. 5b and d) suggests that the basal rate of $V_{cmax}$ (i.e. $V_{cmax}^{25}$) acclimates to $T_{growth}$. Indeed, the decline in Rubisco as a % of N with increasing $T_{growth}$ (Fig. 1e) and the associated fall in Rubisco (Fig. 1a) led to a significant negative linear relationship between $V_{cmax}^{25}$ and $T_{growth}$ (Fig. 6a, Table 1). Part of the decline in $V_{cmax}^{25}$ could be attributed to generally higher $V_{cmax}^{25}$ values in temperate than tropical species, evident in that the $V_{cmax}^{25}$ at $T_{growth}$ of 25°C was higher for temperate than tropical species. Despite the difference at 25°C, the slope and intercept was not significantly different between biomes and a significant negative relationship was evident for the combined analysis (Table 1). As $T_{growth}$ did not significantly influence $N_a$, $V_{cmax}^{25}$ as a proportion of N ($V_{cmax}^{25}$/N) also declined across the entire T-range (Fig. 6b). $J_{max}$ at 25°C ($J_{max}^{25}$) was lower in tropical species than their temperate counterparts, evident in a significant difference in intercept means between biomes (Fig. 6c, Table 1). Similarly, $J_{max}^{25}$/V$_{cmax}^{25}$ were lower in tropical than temperate species and largely independent of $T_{growth}$, with a significant difference in intercept means between biomes (Fig. 5d, Table 1).

**Implications of changing Rubisco content on acclimation-dependent photosynthetic modelling**

From the above analysis, a major factor driving acclimation of photosynthesis is $T_{growth}$-dependent variation in $V_{cmax}^{25}$, underpinned by reduced allocation of leaf N to Rubisco. Yet, $T_{growth}$ driven changes in $V_{cmax}^{25}$ are not factored into previously published Arrhenius and peaked temperature response functions of $V_{cmax}$ (Eqns 5 and 6). Given this, we present a modification to the standard Arrhenius and peaked models, which factors in $T_{growth}$-mediated changes in $V_{cmax}^{25}$ according to:

$$V_{cmax}^{25} = (86 - (1.57 * T_{growth})),$$

where the coefficient term $(86 - (1.57 * T_{growth}))$ incorporates the intercept of $V_{cmax}^{25}$ at 0°C (86 μmol CO$_2$ m$^{-2}$ s$^{-1}$) and $T_{growth}$-dependent decline of $V_{cmax}^{25}$ (1.57 μmol CO$_2$ m$^{-2}$ s$^{-1}$ °C$^{-1}$), calculated from the combined temperate and tropical linear regression analysis of $V_{cmax}^{25}$, as presented in Table 1. Substituting this ‘variable-base’ $V_{cmax}^{25}$ coefficient term into Eqns 5 and 6 provided a good fit ($R^2 = 0.93$ for the Arrhenius and 0.92 for the peaked model) to the observed long-term temperature response of $V_{cmax}$.

---

**Fig. 5** The maximum CO$_2$ carboxylation rates ($V_{cmax}$), for temperate (open blue circles) and tropical (open magenta squares) trees, grown and measured from 15 to 35°C. $V_{cmax}$ was modelled using published Rubisco kinetic parameters of tobacco grown at 25°C. The solid blue and magenta curves are the published tobacco temperature response function of $V_{cmax}$ presented by Bernacchi et al. (2002) and fitted to temperate and tropical species, respectively (with the T response models set to intersect measured values at 25°C). (b) The same as Panel a but with $V_{cmax}$ at each growth temperature adjusted to the Rubisco content measured at 25°C ($V_{cmax}/(\text{Rubisco}_{growth}/\text{Rubisco}_{25°C})$). (c) The percentage deviation of observed relative to expected $V_{cmax}$ ($V_{cmax, \text{observed}}/V_{cmax, \text{expected}}$) at each growth temperature. (d) The same as Panel c but again with values at each growth temperature adjusted to the Rubisco content measured at 25°C. Values are the mean ± standard error of the species means at each growth temperature.
The function shows a diminishing increase in \( V_{c\text{max}} \) with \( T_{\text{growth}} \) above 25°C and an inflection point, where \( V_{c\text{max}} \) reaches a peak, at 40°C. \( V_{c\text{max}} \) values calculated from the variable-base function (Eqn 8) of the Arrhenius and peaked model, were compared with the standard peaked function (Eqn 6), or the standard Arrhenius function (Eqn 5, with iteratively fit \( c \) and \( E_a \) values), and used to predict \( A_n \) values using Eqn 3 (Fig. 7b and c).

\( A_n \) was calculated at each unit of \( T_{\text{growth}} \) from the linear regression analysis of the combined temperate and tropical species response of \( A_n \) to \( T_{\text{growth}} \) (Fig. 3a; Table 1) and \( C_i \) was fixed at a CO\(_2\) partial pressure of 28.8 Pa (based on the assumption of a \( C_i/C_a \) of 0.75 and given that atmospheric pressure at the site of measurements averaged 96 kPa). \( R_{\text{light}} \) was fixed at the combined biome mean \( R_{\text{dark}} \) value of 1.4 \( \mu \)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\), to reduce model extrapolations, considering \( R_{\text{dark}} \) was directly measured from gas-exchange and was similar in values between biomes. The resulting observed \( A_n \) values from gas-exchange and predicted \( A_n \) values using the standard \( V_{c\text{max}} \) models (Fig. 7b), or the models with the variable-base function added (Fig. 7c), were compared (observed values as symbols; predicted values as curved fits). The standard acclimated peaked model and Arrhenius function did not fit at all closely with observed \( A_n \) (Fig. 7b). Without accounting for changes in \( V_{c\text{max}}^{25} \), predicted \( A_n \) was less responsive to temperature, being lower than observed values below 25°C and higher above 25°C. However, the variable-base model predictions (which allows for \( T_{\text{growth}} \)-dependent variations in \( V_{c\text{max}}^{25} \)) did closely fit the observed \( A_n \) values across the entire measured \( T \)-range (Fig. 7c).

**Discussion**

Our study demonstrated that when a set of temperate and tropical wet-forest species experienced a range of growth temperatures similar to that experienced under field conditions, large changes in leaf structure, chemistry and function occurred. Collectively, such patterns point to common thermal acclimation responses of photosynthesis in the selected species from both biomes. Importantly, our study highlights the importance of \( T_{\text{growth}} \)-mediated changes in Rubisco abundance underpinning the acclimation response of the selected species, with the results providing an opportunity to formulate a new \( V_{c\text{max}}^{25} \) temperature response function that accounts for acclimation-dependent changes in \( V_{c\text{max}}^{25} \).

**Photosynthetic and respiratory adjustments to \( T_{\text{growth}} \)**

We observed a linear decline in area-based concentrations of the key photosynthetic enzyme, Rubisco, with...
increasing $T_{\text{growth}}$ (Fig. 1a, Table 1), with these changes in Rubisco content being central to the $T_{\text{growth}}$-dependent changes in $V_{\text{cmax}}$ (Fig. 6) that underpin thermal acclimation responses in the selected tropical and temperate wet-forest species. From the traits measured, we see a number of factors as being responsible for the observed $T_{\text{growth}}$-dependent changes in Rubisco and $V_{\text{cmax}}$. For example, acclimation to increasing $T_{\text{growth}}$ was associated with a decline in LMA (Fig. 1b), an observation consistent with past studies assessing $T_{\text{growth}}$-mediated changes in leaf structure (Poorter et al., 2009). Given that LMA is influenced, in part, by leaf thickness, reduced LMA values will likely be associated with declines in the number and/or size of mesophyll cells (and thus reduced investment in photosynthetic metabolism) per unit leaf area. Yet, despite LMA decreasing with increasing $T_{\text{growth}}$, area-based leaf N did not decline with increasing $T_{\text{growth}}$ (Fig. 1d), reflecting the fact that $N_m$ exhibited a significant positive relationship with $T_{\text{growth}}$ (Fig. 1c). While the covariance of LMA and $N_m$ is consistent with worldwide observations of negative relationships between LMA and $N_m$ (Reich et al., 1997; Wright et al., 2004), in this case it is more interesting in demonstrating that the fraction of total leaf N allocated to Rubisco (Fig. 1d) declined with increasing $T_{\text{growth}}$ (Fig. 1e). In turn, this implies that an increasing fraction of leaf N is allocated to nonphotosynthetic components (e.g. cell wall N and/or defence compounds) as $T_{\text{growth}}$ increases, as has been reported previously for cold and warm acclimated spinach (Yamori et al., 2005). Trade-offs between leaf N investment to Rubisco vs. nonphotosynthetic components are known to occur among species differing in LMA, with high LMA species exhibiting lower relative investment of leaf N in Rubisco (Takashima et al., 2004; Harrison et al., 2009). Interestingly, however, these interspecific comparisons contrast with our observation that the relative N investment in Rubisco was greatest in cold grown, high LMA leaves (Fig. 1). Thus, the direction of the relationship between LMA and N investment seen in multispecies comparisons (largely driven by differences in economic strategies among species) cannot be used to predict $T_{\text{growth}}$-mediated changes in leaf structure and chemistry. In summary, we suggest that thermal acclimation in the temperate and tropical species is underpinned by reduced investment in photosynthetic machinery per unit leaf area that results primarily from a decline in N investment in Rubisco as $T_{\text{growth}}$ increases.

For the declines in Rubisco and $V_{\text{cmax}}$ at high $T_{\text{growth}}$ to have a negative impact on assimilation rates, photosynthesis needs to be largely Rubisco-limited.
Under controlled experimental conditions of saturating light and abundant water supply, we observed $A_c$ limitations at a $C_a$ of 38.4 Pa, irrespective of species, biome-origin and $T_{growth}$ and did not observe $A_r$ limitations until $C_a$ values at close to 58 Pa. Of note, growth irradiance was lower than irradiance during $A_n$ measurements; however tree species growing at low rather than high irradiance have reduced $J_{max}$ relative to $V_{cmax}$ (Niinemets et al., 1998). This would imply that if we did grow trees at higher irradiance, matching that used for photosynthetic capacity measurements, the trees would likely remain Rubisco-limited, and to a greater extent considering $J_{max}$ would likely limit photosynthesis at even higher CO$_2$ partial pressures. With the observed general $A_c$ limitation on photosynthesis, we expected changes in Rubisco content per unit leaf area to directly impact on area-based rates of $A_n$ and $V_{cmax}$. Indeed, we observed a decline in $A_n$ with increased $T_{growth}$ (Fig. 3, Table 1), and the long-term temperature response of $V_{cmax}$ at $T_{growth}$ was shallow when compared to the immediate $V_{cmax}$ temperature response. Importantly, when Rubisco content was standardized across the $T_{growth}$ range (i.e. ignoring any possible acclimation of Rubisco content), variation in $V_{cmax}$ better matched the quasi-exponential $T$ response expected by prior models (Fig. 5), which also ignore acclimation of Rubisco content, further highlighting the importance of $T_{growth}$-mediated changes in Rubisco in the acclimation process. Figure 8 provides a conceptual illustration, summatng our generalized view of how thermal adjustments in Rubisco content impact on photosynthetic capacity.

**Fig. 8** A conceptual illustration of the role of Rubisco content on the short-term response and long-term thermal acclimation of net photosynthesis ($A_n$). The schematic diagram on the left shows the linkage between various photosynthetic carboxylation and respiration components which contribute to $A_n$. The immediate/short-term temperature response of $A_n$ is dependent on: (1) changes in the number of Rubisco active sites, mediated by the Rubisco chaperone protein Rubisco activase (RCA); (2) changes in the catalytic rate constant ($k_{cat}$) influencing the maximum rate of carboxylation ($V_{cmax}$); (3) changes in Rubisco kinetic parameters ($K_c$ and $K_o$) affecting the rate of carboxylation ($V_c$) and photorespiration ($V_o$); (4) CO$_2$ conductance from ambient air ($C_a$) through stomata ($g_s$) and mesophyll cells ($g_m$), affecting CO$_2$ substrate availability of $V_c$ and $V_o$; and (5) short-term responses in light respiration ($R_L$). Thereafter, the long-term thermal acclimation of $A_n$ is dependent on (6) changes in the number of Rubisco active sites, mediated by N content on a leaf area basis ($N_a$) and the % of N allocated to Rubisco; (7) potential adjustments in RCA thermal stability and RCA to Rubisco ratios; and (8) $R_L$ acclimation. A generalized representation of short-term responses and long-term thermal acclimation on $A_n$ for cold grown temperate (15 °C; blue solid lines) and hot grown tropical (35 °C; magenta dashed lines) trees are demonstrated in the right panels, with size of the pie diagrams indicating $N_a$ and the slice representing the percentage of leaf N in Rubisco. Of note, even though Rubisco kinetic properties may not be altered, long-term thermal acclimation results in an increase in $A_n$ for cold grown leaves and decrease in $A_n$ for hot grown leaves at a $C_a$ of 400 μmol mol$^{-1}$, driven by changes in Rubisco content.

An important consequence of the observed acclimation was that the rate of $V_{\text{cmax}}$ at a given leaf N (i.e. photosynthetic N use efficiency) decreased with increasing $T_{\text{growth}}$ underpinned by the reduced allocation of leaf N to Rubisco. An earlier glasshouse comparison of 13 tropical and 12 temperate wet-forest species (Xiang et al., 2013), and field-based studies of tropical and temperate species (Kattge & Knorr, 2009) and cool tropical/montane species (Bahar et al., 2016) all show lower $V_{\text{cmax}}$ and $V_{\text{cmax,N}}$ in wet-forest plants growing in warmer tropical than cooler montane or temperate environments, often attributed to nutrient availability. We see, therefore, a pattern via which low $V_{\text{cmax}}$ and $V_{\text{cmax,N}}$ in warm, tropical wet-forest species is likely due to the combined effects of genotypic (i.e. inherent) and environment-mediated phenotypic responses of individual plants (i.e. acclimation) to shifts in N allocation.

In past studies on thermal acclimation, growth in warm conditions resulted in greater proportional reduction in $I_{\text{max}}$ than $V_{\text{cmax}}$ (Atkin et al., 2006a; Hikosaka et al., 2006; Sage & Kubien, 2007; Yamori et al., 2010) with the result that $I_{\text{max}}/V_{\text{cmax}}$ ratios typically decreased with increasing $T_{\text{growth}}$. Yet, we found that $I_{\text{max}}/V_{\text{cmax}}$ was largely unresponsive to $T_{\text{growth}}$ (Fig. 6), albeit with $I_{\text{max}}/V_{\text{cmax}}$ values being lower in the tropical than temperate trees, as was found by Xiang et al. (2013), evident in different intercept means. While the reasons for the lack of $I_{\text{max}}/V_{\text{cmax}}$ response to $T_{\text{growth}}$ for the selected species remain unclear, it seems possible that $I_{\text{max}}$ may have been overestimated in the temperate species, which were above model expectations (Fig. 4), and gave poor model fits relative to $V_{\text{cmax}}$ (Table 2). The reason for why modelled $I_{\text{max}}$ values did not match expectations, especially for temperate species, needs to be further explored.

We found that rates of $R_{\text{light}}$ were always equal to or greater than $R_{\text{dark}}$ in the temperate and tropical trees (Fig. 3e). On first inspection, this may appear a surprising result, as $R_{\text{light}}$ is typically lower than $R_{\text{dark}}$ – that is light inhibits leaf $R$ (Atkin et al., 2006b; Zaragoza-Castells et al., 2007; Way & Sage, 2008; Scafaro et al., 2012). While there are published examples of where $R_{\text{light}} > R_{\text{dark}}$ (Atkin et al., 2000) – demonstrating that it is possible for respiratory fluxes to be higher in the light than in darkness – estimates of $R_{\text{light}}$ (obtained from $A_{\text{c}}-C_{\text{c}}$ curves) depend on the assumed $\Gamma^*$ value, with error in $\Gamma^*$ leading to concomitant errors in predicted rates of $R_{\text{light}}$. Given this, measurements of $\Gamma^*$ for both temperate and tropical trees should be a priority for future research to resolve the extent to which fluxes of $R_{\text{light}}$ and $R_{\text{dark}}$ differ in trees from these biomes.
expected physiological range will respond to a restricted range of short-term changes in temperature without a $V_{\text{cmax}}$-driven inhibition of $A_c$ photosynthetic capacity. Alternatively, trees experiencing extreme heating events will likely have impaired photosynthetic capacity due to a decline in $V_{\text{cmax}}$ (Medlyn et al., 2002b; Kattge & Knorr, 2007). Together, these findings of stable $V_{\text{cmax}}$—where daily variations in leaf temperature remain relatively close to $T_{\text{growth}}$—and reduced $V_{\text{cmax}}$—where extreme heating events occur—have important implications for the modelling community as they seek to simulate plant CO2 exchange responses to short- and long-term changes in air temperature.

**Implications of thermal acclimation of photosynthesis for the terrestrial biosphere**

In addition to the impact of short-term (minutes to days) shifts in measurement temperature on $V_{\text{cmax}}$, terrestrial biosphere models must consider longer term $V_{\text{cmax}}$ response to temperature (Fig. 7). When considering leaves that had developed in each respective growth temperature, we found that $V_{\text{cmax}}$ exhibited a negative linear relationship with $T_{\text{growth}}$ (Fig. 6). This change in the base rate of $V_{\text{cmax}}$ will shift the Arrhenius function up or down its vertical axis, depending on long-term acclimation temperature. The variable-base model we propose accounts for this change in $V_{\text{cmax}}$ by incorporating the regression value for $V_{\text{cmax}}$ at a given $T_{\text{growth}}$ (Eqn 8). Further refinement of the variable-base model by incorporating the temperature dependent $V_{\text{cmax}}$ of more temperate and tropical species should be a consideration of future work.

We made $A_c$ model predictions using the standard Arrhenius function, the peaked model with acclimation accounted for, or both aforementioned models incorporating a variable-base function (factoring in $V_{\text{cmax}}$ changes), and compared the predictions with actual measurements of photosynthesis at a $C_a$ of 38.4 Pa (Fig. 7b and c). When the variable-base function was included, both models went from poorly predicting the $T$ response of $A_n$, underestimating $A_n$ below 25 °C and overestimating above 25 °C, to being good predictors of observed $A_n$. This suggests that not factoring in the change in Rubisco content and subsequent change in $V_{\text{cmax}}$ with $T_{\text{growth}}$ may lead to an underestimation of forest CO2 uptake in cool climates and overestimation in warmer climates. Indeed, current models do underestimate observed forest CO2 uptake in cooler temperate and boreal biomes and overestimate tropical biome CO2 uptake, attributed to not factoring in differences in $V_{\text{cmax}}$ between PFTs (Kattge et al., 2009), and consistent with our findings. Of note, we observed $R_{\text{dark}}$ and $R_{\text{light}}$ acclimation, leading to homeoestasis of respiration in darkness and in the light across the entire 15–35 °C range. Respiratory homoeostasis reduced the amount of CO2 lost as a proportion of photosynthetic capacity for species grown at higher temperatures, matching global observations (Atkin et al., 2015) and recent field-warming experiments (Aspinwall et al., 2016; Drake et al., 2016; Reich et al., 2016). Hence, respiratory acclimation will compensate for some of the carbon loss resulting from photosynthetic capacity downregulation and improve energy use efficiency of trees growing in a warmer world. Moreover, the demonstration from our study and the three above cited field experiments of rising respiration : photosynthesis ratios at warmer growth temperatures, despite homeoestatic or near-homeoestatic temperature acclimation of dark respiration, suggests that parameterizing respiration in models as a fixed proportion of $V_{\text{cmax}}$ is likely inappropriate.

The reason for why there is a general trend of photosynthetic downregulation in response to $T_{\text{growth}}$ is an intriguing question. Considering that we maintained consistent water availability and there was no significant correlation between $T_{\text{growth}}$ and $g_s, C_i$ or $C_r$ (Fig. 3c, d), we do not attribute the downregulation in photosynthesis as a response to CO2 diffusion limitations or water stress. Reduced photosynthetic capacity in response to warmer $T_{\text{growth}}$ may be a regulatory mechanism to balance the temperature effects on assimilate source and sink capacity (Fatichi et al., 2014). Similarly, plants grown under enriched ambient CO2 alter photosynthetic capacity in response to source/sink limitations (Ainsworth et al., 2003; Long et al., 2004; Ainsworth & Long, 2005; Rosenthal et al., 2012).

In conclusion, the $V_{\text{cmax}}$ temperature response of thermally acclimated trees from 15 to 35 °C displayed a standard exponential Arrhenius function, with no observable acclimation in Rubisco kinetic properties to temperature. However, the basal rate of $V_{\text{cmax}}$ (i.e. $V_{\text{cmax}}$) fell with long-term acclimation to warmer $T_{\text{growth}}$, due to a concomitant fall in Rubisco content per unit leaf area, driven by lower N allocation to Rubisco. The result was a downward shift in photosynthesis when leaves develop in warmer environments. If these findings hold for natural ecosystems, then one might expect trees to display lower rates of photosynthesis per unit leaf area in a warmer world, regulated by a disinvestment in Rubisco.

**Acknowledgements**

This work was supported by the Overseas Foundation of the Chinese Academy of Sciences and National Natural Science Foundation of China (31000290, 31370594) and International Science Cooperation Program of China (2013DFR00670) to SX, and Australian Research Council support (FT0991448, DP0986823, DP1093759 and CE140100008 to OKA).


Sage RF (2002) Variation in the k\textsubscript{cat} of Rubisco in C\textsubscript{3} and C\textsubscript{4} plants and some implications for photosynthetic performance at high and low temperatures. *Journal of Experimental Botany*, 53, 609-620.


