Acclimation of leaf respiration consistent with optimal photosynthetic capacity

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Running Title (A short running title of less than 45 characters including spaces)
Optimal acclimation of leaf dark respiration
Abstract

Plant respiration is an important contributor to the proposed positive global carbon-cycle feedback to climate change. However, as a major component, leaf mitochondrial (‘dark’) respiration ($R_d$) differs among species adapted to contrasting environments and is known to acclimate to sustained changes in temperature. No accepted theory explains these phenomena or predicts its magnitude. Here we propose that the acclimation of $R_d$ follows an optimal behaviour related to the need to maintain long-term average photosynthetic capacity ($V_{cmax}$) so that available environmental resources can be most efficiently used for photosynthesis. To test this hypothesis, we extend photosynthetic co-ordination theory to predict the acclimation of $R_d$ to growth temperature via a link to $V_{cmax}$, and compare predictions to a global set of measurements from 112 sites spanning all terrestrial biomes. This extended co-ordination theory predicts that field-measured $R_d$ should increase by 3.7% and $V_{cmax}$ by 5.5% per degree increase in growth temperature. These acclimated responses to growth temperature are less steep than the corresponding instantaneous responses, which increase 8.1% and 9.9% per degree of measurement temperature for $R_d$ and $V_{cmax}$, respectively. Data-fitted regression slopes proof indistinguishable from the values predicted by our theory, and smaller than the instantaneous slopes. Theory and data are also shown to agree that the basal rates of both $R_d$ and $V_{cmax}$ assessed at 25°C decline by ~ 4.4% per degree increase in growth temperature. These results provide a parsimonious general theory for $R_d$ acclimation to temperature that is simpler – and potentially more reliable – than the plant functional type-based leaf respiration schemes currently employed in most ecosystem and land-surface models.

Keywords (6-10)

acclimation, carbon cycle, carboxylation capacity ($V_{cmax}$), climate change, co-ordination, land-surface model, leaf mass per area, leaf nitrogen, nitrogen cycle, optimality, photosynthesis
1 | Introduction

Land plant respiration is a major component of the carbon cycle, releasing ca. 60 Pg C yr\(^{-1}\) to the atmosphere: six times more than anthropogenic CO\(_2\) emissions from all sources combined (Ciais et al., 2014). About half is due to mitochondrial respiration in leaves (Atkin et al., 2007), which is usually called ‘dark’ respiration (\(R_d\)) since it is most easily measured in darkened leaves; mitochondrial respiration continues in the light, although at a reduced rate (Tcherkez et al., 2017). Leaf respiration is closely coupled with photosynthetic activity (Hoefnagel et al., 1998, Noguchi & Yoshida, 2008, O’Leary et al., 2019, Tcherkez, 2012, Wright et al., 2004). As described by the standard biochemical model of photosynthesis (Farquhar et al., 1980), the instantaneous rate of photosynthesis by C\(_3\) plants is limited either by the capacity of the enzyme Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) for the carboxylation of RuBP (\(V_{cmax}\)), or by the rate of electron transport for the regeneration of RuBP, which depends on absorbed light and the electron transport capacity (\(J_{max}\)). \(R_d\) of fully developed leaves is used to support metabolic processes including protein turnover, phloem loading, the maintenance of ion gradients between cellular compartments, nitrate reduction, and the turnover of phospholipid membranes. Among these, protein turnover is the largest contributor to variation in \(R_d\). Given that Rubisco is a substantial fraction of total leaf protein, \(R_d\) is expected to scale closely with \(V_{cmax}\), which sets the daily maximum photosynthetic rate achieved by leaves under natural growing conditions (Amthor, 2000, Atkin et al., 2000, Bouma, 2005, Cannell & Thornley, 2000, O’Leary et al., 2019). \(R_d\) is commonly assumed in Land Surface Models (LSMs) to be proportional either to \(V_{cmax}\) or, alternatively, to area-based leaf nitrogen content (\(N_{area}\)) (Rogers, 2014).

Leaf respiration is enzyme-catalysed and therefore temperature-dependent. On a time scale of minutes to hours, \(R_d\) responds to leaf temperature near-exponentially and is determined principally by the temperature dependence of the reaction rates of multiple enzymes involved in various respiratory pathways in the cytosol and mitochondria (Atkin et al., 2005, Atkin & Tjoelker, 2003). We refer to this observed, composite temperature response as the “instantaneous” response. Because of this temperature response of \(R_d\), it has been proposed that global warming will increase plant respiration and accelerate climate change (Cox et al., 2000, Huntingford et al.,...
However, the magnitude of this positive feedback remains unclear. It depends on the sensitivity of plant respiration to temperature changes over longer time scales, which—as many experiments have shown—is damped, relative to the short-term response, by acclimation (Atkin & Tjoelker, 2003, Reich et al., 2016, Scafaro et al., 2017). The longer-term response of plant respiration to the prevailing growth temperature is also manifest in spatial patterns of leaf $R_d$ (Atkin et al., 2015, Slot & Kitajima, 2015), which show a far less steep pole-to-equator gradient than would be expected from the instantaneous response—a consequence of both acclimation (plastic responses) and adaptation, i.e. differences among genotypes and species adapted to contrasting environments. Vanderwel et al. (2015) moreover demonstrated consistency between the observed spatial pattern of $R_d$ and the acclimation of leaf $R_d$ over time. Pervasive long-term acclimation of respiration implies a weaker positive carbon-climate feedback than is implied by the instantaneous temperature response (Huntingford et al., 2017, Reich et al., 2016, Smith et al., 2016). Neglecting the acclimation of plant respiration to temperature in LSMS may therefore be a major source of bias in Earth System model predictions (Huntingford et al., 2017, Smith et al., 2016).

Quantitative explanations and predictions of the acclimation and adaptation of leaf $R_d$ to temperature are still lacking. Conclusions from empirical studies alone (Wright et al., 2006) are insufficient to address the underlying causality; a firmer theoretical basis is essential to build confidence in carbon-cycle predictions (Prentice et al., 2015). Here we propose a theoretical framework for the acclimation of leaf $R_d$ based on a few key hypotheses. We first assume acclimation of $V_{c_{\text{max}}}$ (Step 1 in Figure 1) via the ‘co-ordination hypothesis’, which states that $V_{c_{\text{max}}}$ assessed at growth temperature ($V_{c_{\text{max,tg}}}$) has a general tendency to adjust to average daytime conditions so that the electron transport- and Rubisco-limited photosynthetic rates ($A_J$, $A_c$) are co-limiting (Chen et al., 1993, Haxeltine & Prentice, 1996, Maire et al., 2012) (that is, $A_J \approx A_c$). Co-limitation is optimal in an eco-evolutionary sense because any other outcome would either incompletely exploit available light, or require additional respiration to maintain excess amounts of Rubisco. This hypothesis explained 64% of field-measured $V_{c_{\text{max,tg}}}$ variability in C$_3$ plants across different biomes, and has been used with success to predict global patterns of primary production (Smith et al., 2019a, Wang et al., 2017b). Second, the various metabolic functions of $R_d$ in mature leaves...
are assumed to be tightly coupled to $V_{\text{cmax}}$ (Step 3 in Figure 1) – implying a close link between the acclimation of $V_{\text{cmax}}$ and $R_d$. We test this hypothesis later, alongside the alternative hypothesis that $R_d$ depends on $N_{\text{area}}$. To simplify the theoretical framework and mathematical derivations, we (a) disregard any possible differences in the instantaneous thermal responses of $R_d$ and $V_{\text{cmax}}$ among species and across sites (Steps 2 and 4 in Fig 1), and (b) assume infinite mesophyll conductance and non-limiting electron-transport capacity (Keenan et al., 2016, Togashi et al., 2018, Wang et al., 2014). Although uncertainties are thereby inevitably introduced, these simplifications allow us to test first-order effects at a global scale, appropriately for the potential improvement of LSMs.

2 | Materials and methods

2.1 | Quantitative predictions

Based on the simplifying assumption that leaf $R_d$ adjusts over time primarily to maintain the turnover of Rubisco and other enzymes involved in the Calvin cycle, we start from the premise that at the prevailing growth temperature ($T_g$), the acclimated $R_{d,t_g}$ is proportional to acclimated $V_{\text{cmax,t_g}}$:

$$R_{d,t_g} = b_{t_g} V_{\text{cmax,t_g}}$$  (1)

while recognizing that the proportionality factor $b_{t_g}$ could vary with environmental conditions. We therefore first focus on quantitative prediction of the optimal thermal acclimation of $V_{\text{cmax,t_g}}$.

2.1.1 | Step 1: optimal $V_{\text{cmax,t_g}}$ and its thermal acclimation based on the coordination hypothesis

We hypothesize that $V_{\text{cmax}}$ of leaves at any canopy level acclimates to the current environment in such a way that the Rubisco-limited (increasing with $V_{\text{cmax}}$) and electron transport-limited (increasing with absorbed PPFD) photosynthetic rates tend to converge. This is the ‘strong form’ of the coordination hypothesis (Chen et al., 1993, Haxeltine & Prentice, 1996, Maire et al., 2012), contrasting with a ‘weak form’ that assumes that the total metabolic N content of the leaf is prescribed so that only the allocation of N to carboxylation versus electron transport capacities is optimized (e.g. Quebbeman and Ramirez (2016)). In response to environmental variations, the
coordination hypothesis predicts vertical variation of \( V_{\text{cmax,fg}} \) within the canopy, geographic variation among sites, and temporal variations with atmospheric CO\(_2\) concentration and climate (Haxeltine & Prentice, 1996, Smith et al., 2019b, Terrer et al., 2018). Thus, under field conditions the coordination hypothesis predicts that (Wang et al., 2017a):

\[
V_{\text{cmax,fg}} \approx \varphi_0 I_{\text{abs}}(\chi c_a + K) / (\chi c_a + 2\Gamma^*)
\]  

where \( \varphi_0 \) is the intrinsic quantum efficiency of photosynthesis (mol mol\(^{-1}\)); \( I_{\text{abs}} \) is the PPFD absorbed by the leaf (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)); \( \chi \) is the ratio of leaf-internal to ambient partial pressure of CO\(_2\) (Pa Pa\(^{-1}\)); \( c_a \) is the ambient partial pressure of CO\(_2\) (Pa); \( \Gamma^* \) is the photorespiratory compensation point (Pa); and \( K \) is the effective Michaelis-Menten coefficient of Rubisco (Pa). \( \Gamma^* \) and \( K \) are temperature-dependent following Arrhenius relationships as measured e.g. by Bernacchi et al. (2001). Acknowledging that Rubisco kinetics traits vary both within and among species, we applied various Rubisco catalytic constants (the Michaelis–Menten coefficients for carboxylation and oxygenation, and the Rubisco specificity factor) provided by (Galmés et al., 2016) to estimate the uncertainties (± 1 s.d.) in \( K \), \( \Gamma^* \) and their instantaneous thermal responses.

The least-cost hypothesis (Prentice et al., 2014, Wang et al., 2017b) predicts optimal \( \chi \) to be a function of growing-season mean values of temperature (\( T_g \); K), vapour pressure deficit (\( D \); Pa) and elevation (\( z \); m). These predictions are quantitatively supported by worldwide measurements of \( \chi \) across species and biomes (Wang et al., 2017b). Equation (2) then yields estimates of \( V_{\text{cmax}} \) given \( \chi \) and field-relevant average values of \( c_a \) (Pa), temperature (K) and PPFD (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)).

We define temperature sensitivities (\( \beta \)) of various quantities as fractional increases per degree. The fractional sensitivity of \( V_{\text{cmax,fg}} \) to temperature after acclimation (\( \beta_{aV} \)) can be deduced by differentiating equation (2) with respect to \( T_g \):

\[
\beta_{aV} = \left( \frac{\partial V_{\text{cmax,fg}}}{\partial T_g} \right) / V_{\text{cmax,fg}} = \frac{\partial \ln V_{\text{cmax,fg}}}{\partial T_g}
\]

\[
= \frac{\partial \ln (\chi c_a + K)}{\partial T_g} - \frac{\partial \ln (\chi c_a + 2\Gamma^*)}{\partial T_g} (3)
\]

Evaluating equation (3) under standard conditions (\( T_g = 298 \) K, \( D = 1 \) kPa, \( z = 0 \), \( c_a = 40 \) Pa) yields \( \beta_{aV} = 5.5 \pm 0.3 \% \text{ K}^{-1} \). This value derives primarily from the sensitivities
of $K$ and $I^*$ to temperature (8.5% K$^{-1}$ and 5.4% K$^{-1}$, respectively), which depend on their activation energies (Bernacchi et al., 2001), and to a lesser extent from the sensitivity of $\chi$ to temperature (0.9% K$^{-1}$).

### 2.1.2 | Step 2: optimal $V_{\text{cmax,25}}$ and its thermal acclimation

Described by a modified Arrhenius function (Kattge & Knorr, 2007), the instantaneous temperature response of $V_{\text{cmax}}$ to temperature provides a link between $V_{\text{cmax,tg}}$ and $V_{\text{cmax,25}}$:

\[ V_{\text{cmax,tg}} = V_{\text{cmax,25}} \times f_v \]

where

\[ f_v = e^{H_a (T_g-298.15) / 298.15 T_g R} \times \left[ 1+e^{(298.15AS - H_d) / (298.15 R)} \right] / \left[ 1+e^{(T_g AS - H_d) / (T_g R)} \right] \]

where $H_a$ is the activation energy (71 513 J mol$^{-1}$), $R$ is the universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$), $T_{\text{ref}} = 298.15$ K, $H_d$ is the deactivation energy (200 000 J mol$^{-1}$), and $\Delta S$ is an entropy term (J mol$^{-1}$ K$^{-1}$), which can be calculated using a linear relationship with $T_g$ from (Kattge & Knorr, 2007) with a slope of 1.07 J mol$^{-1}$ K$^{-2}$ and an intercept of 668.39 J mol$^{-1}$ K$^{-1}$.

To estimate the uncertainties ($\pm$ 1 s.d.) in Rubisco kinetics, we applied various maximum carboxylase turnover rates provided by Galmés et al. (2015). Equation (4) then generates an instantaneous response of $V_{\text{cmax}}$ to temperature with a sensitivity $\beta_{iv}$ of 9.9 $\pm$ 1.4 % K$^{-1}$, and allows $\beta_{qV}$ to be derived as:

\[ \beta_{qV} = \partial \ln V_{\text{cmax,25}} / \partial T_g \]

\[ = \partial \ln V_{\text{cmax,tg}} / \partial T_g - \partial \ln f_v / \partial T_g \]

\[ = \beta_{aV} - \beta_{iv} \]

\[ = -4.4 \pm 1.4 \text{ % K}^{-1} \]

We can thus break down the acclimated temperature sensitivity of $V_{\text{cmax,tg}}$ ($\beta_{aV}$) into the instantaneous sensitivity of Rubisco to temperature changes ($\beta_{iv}$) and the acclimated sensitivity ($\beta_{qV}$) of the amount of Rubisco (as indexed by $V_{\text{cmax,25}}$, the catalytic activity of Rubisco at 25°C) to growth temperature.
2.1.3 | Step 3: optimal $R_{d,25}$ and its thermal acclimation based on the link to $V_{cmax,25}$

In commonly used photosynthesis models, leaf $R_{d,25}$ is assumed proportional to $V_{cmax,25}$ with the ratio given as 0.011 (Farquhar et al., 1980) or 0.015 (Collatz et al., 1991). This assumption implies that $R_{d,25}$ is related to $V_{cmax,25}$ by a constant factor $b_{25}$:

$$R_{d,25} = b_{25} V_{cmax,25} \quad (6)$$

and thus also that $\beta_{qV} = \beta_{qR}$ (Figure 1).

We test this key assumption in parallel empirical analyses. The effects of other potential influences, including leaf mass per area, leaf nitrogen content and soil properties, on $R_{d,25}$ are also tested.

2.1.4 | Step 4: optimal $R_{d,gt}$ and its thermal acclimation

Heskel et al. (2016) provided an empirical formula to estimate $R_d$ at 25°C:

$$\ln R_{d,25} = a + 0.1012 \times 25 - 0.0005 \times 25^2 \quad (7)$$

where $a$ is an empirical constant varying among biomes, representing the natural logarithm of the value of $R_d$ extrapolated to 0°C. The instantaneous response of $R_d$ to temperature ($\beta_{IR}$) as given by Heskel et al. (2016) is 8.1% K$^{-1}$ at the mean $T_g$ of the data. $\beta_{IR}$ is slightly smaller than $\beta_{IV}$, and leads to a response of parameter $b_{tg}$ in equation (1) given by the difference between $\beta_{IR}$ and $\beta_{IV}$ ($\beta_b = -1.8\%$ K$^{-1}$). This then generates the predictions $\beta_{qR} = -4.4\%$ K$^{-1}$ and $\beta_{aR} = 3.7\%$ K$^{-1}$.

Using other instantaneous thermal response curves (for example, equations 1 and 3 in Atkin et al. (2015), equation 1 in Reich et al., 2016), and equation 1 in Kattge and Knorr (2007) yielded slightly different instantaneous responses of $R_d$ and $V_{cmax}$ to temperature. However, those changes also effect the temperature adjustment we applied in the parallel empirical analysis, and have little influence on our testing. We therefore report only the results from the equations as described above.
2.2 | Empirical analyses

2.2.1 | Photosynthesis and respiration data

We combined two $R_d$ datasets: the global respiration (GlobResp) and leaf carbon exchange (LCE) datasets. GlobResp (Atkin et al., 2015) contains measurements of leaf $R_d$, $V_{cmax}$, $N_{area}$ and leaf mass per area (LMA) from 899 species at 100 locations across the major biomes and continents, including data from an earlier compilation by Wright et al. (2004). LCE (Smith & Dukes, 2017a) contains field measurements of leaf carbon exchange and chemical traits from 98 species at 12 locations spanning 53° latitude in North and Central America (Figure S1). Replicated measurements in LCE on the same species and site were averaged. Juvenile samples were excluded. Leaf $R_d$ measurements in both datasets followed the same protocol. Both were taken on fully expanded leaves in daytime after a period of dark adjustment. $V_{cmax}$ values in GlobResp were estimated by the ‘one-point method’ (De Kauwe et al., 2016) whereas those in LCE were estimated from full $A-c_i$ curves. With a global dataset of $A-c_i$ curves (564 species from 46 field sites, covering a range of plant functional types), De Kauwe et al. (2016) showed that ‘the one-point method’ can provide a robust approach to expand the available set of field measurements on $V_{cmax}$. We present analyses based on the combined datasets as the main results in this paper. However, given that Burnett et al. (2019) recently showed that the one-point method may underestimate $V_{cmax}$, we also analysed each dataset separately. The results are given in the Supplementary Information.

We indexed $T_g$ by the mean temperature during the thermal growing season with temperatures above 0°C (mGDD$_0$) (Harrison et al., 2010). $V_{cmax}$ and $R_d$ values in both datasets were provided with information about measurement leaf temperatures. $V_{cmax}$ and $R_d$ values were adjusted both to mGDD$_0$ and to 25°C using the relevant instantaneous responses, as given in Heskel et al. (2016) and Kattge and Knorr (2007), respectively.

A global climatology of monthly temperature provided by the Climatic Research Unit at a grid resolution of 10 arc minutes (CRU CL2.0) (New et al., 2000) was used to provide estimates of mGDD$_0$ for each location. Thermal acclimation of $R_d$ should in principle apply to both C$_3$ and C$_4$ plants, but our theoretical prediction of $V_{cmax}$

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acclimation here is developed for C_3 plants, and we did not include C_4 species in our analysis.

2.2.2 | Statistical analysis

The theoretical framework proposed here includes a series of quantitative predictions. Statistical analysis focused on testing the agreement between these theoretical predictions and data. To test our predictions of $\beta_a$ and $\beta_q$ quantitatively, the $R_d$ and $V_{\text{cmax}}$ data (assessed at mGDD₀ and 25°C) were first normalized with estimates of the site-mean PPFD absorbed by leaves (PPFD_L: see Dong et al. (2017b)) before performing Ordinary Least Squares (OLS) regression against growth temperature. This normalization is appropriate because $V_{\text{cmax}}$ is both predicted (equation 2) and observed (Niinemets & Keenan, 2012) to vary in proportion to PPFD. If it were omitted, the positive effect of PPFD on $R_d$ and $V_{\text{cmax}}$ would contribute to the fitted slope of mGDD₀ due to the correlation between those two variables (Figure S2).

PPFD_L was devised to deal with the fact that field-measured photosynthetic trait data reflect leaves developed at a range of irradiances at different levels in the canopy. PPFD_L is estimated from growing-season total incident PPFD at the top of the canopy (PPFD₀) as follows:

$$PPFD_L \approx f \frac{PPFD_0}{L}$$

(8)

where $f$ is the fraction of incident PPFD absorbed by the canopy (obtained from SeaWiFS data (Gobron et al., 2006)) and $L$ is the leaf area index estimated from Beer’s law:

$$L \approx -\frac{1}{k} \ln (1 - f)$$

(9)

with $k = 0.5$ (Dong et al., 2017a). PPFD₀ was calculated from CRU CL2.0 data using the SPLASH model (Davis et al., 2017).

We applied OLS linear regression of normalized and natural log-transformed $R_d$ and $V_{\text{cmax}}$ values against mGDD₀ using all-species and site-mean data, respectively. To check the impact of the PPFD normalization, we also performed regressions without it. We additionally applied mixed-effects models with species or sites contributing random effects. To test the uncertainty introduced by applying a single set of
instantaneous responses, whereas different kinetics responses might arise among species and sites, we conducted a further OLS regression by using a subset of the dataset when $R_d$ and $V_{cmax}$ were measured at a leaf temperature that differs from 25°C or growth temperature less than 1°C.

To test the key assumption that $R_{d,25}$ is mainly determined by $V_{cmax,25}$, we applied OLS linear regression of $R_d$ versus $V_{cmax}$ (standardized to 25°C and separately to mGDD$_0$, without transformation) to estimate $b_{25}$ and $b$ directly from the fitted slopes. We also included LMA and soil pH as additional predictors in the regression described above. LMA carries information on the structural component of plant leaves. Broadly speaking, higher soil pH and cation exchange capability indicate higher soil fertility (Jenny, 1994, Sinsabaugh & Follstad Shah, 2012), and pH has been shown to influence $\chi$ (Wang et al., 2017b). These covariates were selected to test any potential influences of leaf structure and soil nutrient availability on $R_{d,25}$. An estimate of soil pH for each location was extracted from the Harmonized World Soil Database (http://www.iiasa.ac.at/web/home/research/researchPrograms/water/HWSD.html).

Relationships of $N_{area}$ with $V_{cmax}$ and $R_d$ were also tested by OLS linear regression with or without LMA as an additional predictor. All regressions were performed in R (version 3.5.1).

3 | Results

3.1 | Testing the theoretical implications: thermal acclimation of $R_d$, $V_{cmax}$ and their ratio

The predicted relationship of $V_{cmax,tg}$ to growth temperature ($\beta_{AV}$) was 5.5% K$^{-1}$ under standard environmental conditions. The difference between the instantaneous sensitivities of leaf $R_d$ and $V_{cmax}$ to temperature implies that the sensitivity of acclimated $R_{d,tg}$ to temperature ($\beta_{AR}$) is 1.8% lower than that of $V_{cmax,tg}$ ($\beta_{AV}$), implying a theoretical optimum rate of increase of $R_{d,tg}$ by 3.7% K$^{-1}$. Despite the various simplifications we made, and large variation among species at any given site, these theoretical values are very close to the data-fitted values – whether obtained from all-species or site-mean analyses. They are however shallower than the 9.9% and 8.1% K$^{-1}$ predicted for the instantaneous responses of $V_{cmax}$ and $R_d$ (Table 1, Figure 2). In the all-species analysis, in spite of the large within-site spread, our theoretical
predictions are indistinguishable from the fitted regression coefficients of normalized and transformed $V_{cmax,tg}$ and $R_{d,tg}$ against mGDD$_0$ (5.2 ± 0.3% K$^{-1}$, $R^2 = 0.48$ for $V_{cmax,tg}$, and 3.3 ± 0.2% K$^{-1}$, $R^2 = 0.34$ for $R_{d,tg}$) (Table 1, Figure 2). Growth temperature alone explains 45% and 65% of the observed site-mean variation in $V_{cmax,tg}$ and $R_{d,tg}$ respectively (Table S1, Figure S3).

Theoretically predicted values of the fractional sensitivities of acclimated $R_{d,25}$ ($\beta_{aR25}$) and $V_{cmax,25}$ ($\beta_{aV25}$) to temperature are negative (−4.4 % K$^{-1}$) and this is consistent with the observed negative responses of $R_{d,25}$ and $V_{cmax,25}$ to temperature seen in the data (Table 1). The observed negative response of $V_{cmax,25}$ to growth temperature is indistinguishable (−4.2 ± 0.3% K$^{-1}$ for all-species, −3.4 ± 0.9% K$^{-1}$ for site-mean) from our theoretical prediction, while the observed response of $R_{d,25}$ is marginally larger than our all-species prediction (−4.9 ± 0.3% K$^{-1}$), but indistinguishable in the site-mean analysis (−4.3 ± 0.7% K$^{-1}$).

Regressions performed without PPFD-normalization showed temperature responses with the same signs (positive for $V_{cmax}$ and $R_d$ at growth temperature, negative at 25°C) but slightly steeper (positive slopes) or shallower (negative slopes) than in the main analyses (Table S2) – as expected due to the confounding of PPFD and temperature effects (Figure S2), which normalization removes. $R^2$ values were consistently greater in the main analyses, by 6-7% for $V_{cmax}$ and $R_d$ at growth temperature and 11-17% for $V_{cmax}$ and $R_d$ at 25°C.

Mixed-effects models show a similar acclimation pattern as the OLS regression models (Table S3). When the random effect from either species or site effect is included, the predicted positive (but weaker than instantaneous) thermal responses of $R_{d,tg}$ and $V_{cmax,tg}$ emerge from the data. The resulting negative thermal responses of $R_{d,25}$ and $V_{cmax,25}$ are also supported by the data. The data-fitted thermal sensitivities after acclimation show patterns in agreement with our theoretical predictions ($\beta_{aR} < \beta_{aV}$ whereas $\beta_{aR25} = \beta_{aV25}$), although the fitted sensitivities in these analyses are marginally higher than the theoretical predictions (Table S3).

Using only a subset of the dataset with no temperature correction applied to the measured $R_d$ and $V_{cmax}$, we again show thermal acclimation of $R_{d,25}$ and $V_{cmax,25}$ consistent with our prediction. The responses of $R_{d,gt}$ and $V_{cmax,gt}$ to growth
temperature in this analysis are stronger than predicted, but the uncertainties are much larger due to the limited size of this subset (Table S4).

Regressions based on the GlobResp and LCE datasets separately are generally consistent with our theoretical predictions (Table S5). The LCE dataset shows a stronger acclimation in $R_{d,\text{tg}}$ and a weaker acclimation in $R_{d,25}$ than $V_{\text{cmax}}$ ($\beta_{aR} > \beta_{aV}$, $\beta_{aR25} < \beta_{aV2}$), but the empirically estimated sensitivities are nevertheless close to our theoretical predictions.

The prediction that $b$ should decline with temperature by 1.8% K$^{-1}$ was consistent with the fitted regressions of the ratio of $R_{d,\text{tg}}$ to $V_{\text{cmax,\text{tg}}}$; we observed a small but significant negative response of $b$ to growth temperature with a sensitivity of 2.0% ± 0.3%, while $b_{25}$ was indeed independent of mGDD$_0$ (Table 1, Table S3, Figure 2). The fitted temperature response of $R_{d,\text{tg}}$ was consistently about 2% less steep than that of $V_{\text{cmax,\text{tg}}}$ (Table 1). However, the observed temperature-dependence of this ratio is weak and becomes non-significant in the analyses of site-mean data or the LCE dataset alone (Table S1, S3, S5).

### 3.2 | Testing the theoretical assumptions: relationships of dark respiration and photosynthetic capacity to other variables

We examined the relationships between $R_d$, $V_{\text{cmax}}$ and other potential influences, in order to further test our assumption that among those variables $R_d$ is most strongly correlated to variations in $V_{\text{cmax}}$. We found that measured $R_d$ and $V_{\text{cmax}}$ were positively correlated in the datasets when normalized either to mGDD$_0$ ($R^2 = 0.25$) or to a reference temperature of 25°C ($R^2 = 0.16$) (Table S6). The canonical value of $b_{25} = 0.015$ in the photosynthesis model of Collatz et al. (1991), was similar to the fitted value of $b_{25} = 0.014 \pm 0.001$ based on the regression of $R_{d,25}$ with respect to $V_{\text{cmax},25}$ (Table S6). The inclusion of LMA or soil pH in addition to mGDD$_0$ as a predictor provided negligible increases in explained variance (Table S7).

Relationships of leaf $R_d$ and $V_{\text{cmax}}$ to $N_{\text{area}}$ were similar in strength when normalized to 25°C ($R^2 = 0.14$ and 0.12) (Table 2), but notably weaker when considered at growth temperature ($R^2 = 0.05$ for $R_{d,\text{tg}}$ and 0.02 for $V_{\text{cmax,\text{tg}}}$). LMA and $V_{\text{cmax},25}$ together accounted for 42% variation in $N_{\text{area}}$, but most of this explanatory power comes from
LMA (Table 2). LMA and \( R_{d,25} \) together explained 41\% variation in \( N_{\text{area}} \), but again most of this explanatory power is due to LMA (Table 2).

4 | Discussion

4.1 | Comparison with other studies

Heskel et al. (2016) provided an empirical function for leaf \( R_d \) at 25°C (equation (4) in Methods), where the parameter \( a \) (the logarithm of the basal rate of \( R_d \) at 0°C) is −1.60 for tundra, declining to −2.75 for lowland tropical rainforest. We estimated \( a \) by rearranging equation (3) in Heskel et al. (2016) at a reference temperature of 25°C, and assuming proportionality between \( R_{d,25} \) and \( V_{\text{cmax,25}} \), yielding independent estimates: \( a = -1.41 \) for tundra, and −2.50 for lowland tropical rainforest. The values of \( a \) given by Heskel et al. (2016) allow us to approximate the thermal sensitivity of \( a \) as −4.6\% K\(^{-1}\), assuming a growth temperature range of 25°C from tundra to rainforest: close to our prediction, \( \beta_{aR} = -4.4\% \) K\(^{-1}\). Our results are also consistent with previous findings showing that while \( V_{\text{cmax,lg}} \) increases with growth temperature, \( V_{\text{cmax,25}} \), the amount of Rubisco, and the fraction of leaf N allocated to Rubisco all decline (Scafaro et al., 2017).

The canonical ratio \( R_{d,25}/V_{\text{cmax,25}} = 0.015 \) (Collatz et al., 1991) perhaps co-incidentally lies within the 95\% confidence intervals of the fitted slope (0.014 ± 0.001) obtained by regression of \( R_{d,25} \) on \( V_{\text{cmax,25}} \) (Table S6). We found \( R_{d,25}/V_{\text{cmax,25}} \) was not significantly related to growth temperature but a small significant negative response of \( R_{d,lg}/V_{\text{cmax,lg}} \) to growth temperature (2.0\% ± 0.3\% K\(^{-1}\)) (Figure 2). Our theory predicts a temperature dependence of the ratio of \( R_{d,gt} \) to \( V_{\text{cmax,gt}} \) due to their different instantaneous thermal responses, and this is observed, but the relationship to temperature is much less robust than that of \( R_d \) and \( V_{\text{cmax}} \) themselves.

4.2 | Implications of photosynthetic and respiratory acclimation

We predicted that field-measured \( V_{\text{cmax,lg}} \) and \( R_{d,lg} \) should increase with growth temperature by 5.5\% and 3.7\% per degree, respectively (Table 1, Figure 2). These responses are not instantaneous biochemical responses. They arise, instead, because of the differential temperature sensitivities of two quantities – the effective Michaelis-Menten coefficient of Rubisco (\( K \)) and the photorespiratory CO\(_2\) compensation point.
(I^*) (see equation 3 in Methods). The acclimated responses are determined by the coordination between the Rubisco-limited and light-limited photosynthesis rates, and achieved by the changes of the amount of Rubisco. These predicted thermal sensitivities ($\beta_{aV}$, $\beta_{aR}$) are within the 95% confidence intervals of regression coefficients independently derived from data (Table 1, Figure 2). The tundra site from Alaska with growth temperature around 5°C is dominated by C_3 herbaceous and shrub species, which show high $R_{d,tg}$ values comparable or even higher than the tropical sites. Although the normalization by its low level of site-mean PPFD_L contributes to the high $R_{d,tg}$ values in Figure 2, the observed $R_{d,tg}$ values without normalization are also higher than other sites with similar growth temperature (Figure S4). Meanwhile, the observed $V_{cmax,tg}$ from this site seems quite comparable to other sites. This decoupling might indicate some other energy consuming processes in leaves of those species from extreme environmental conditions.

Many ecosystem and land-surface models disregard acclimation, and assume that the long-term $R_d$ and $V_{cmax}$ responses to temperature follow the instantaneous functions routinely observed ($\beta_{iV} = 9.9% K^{-1}$ (Kattge & Knorr, 2007) and $\beta_{iR} = 8.1% K^{-1}$ (Heskel et al., 2016). Our results contradict this assumption, and provide a quantification of the temperature responses of both $V_{cmax}$ and $R_d$ that explicitly takes acclimation into account. Given that $V_{cmax}$ has been found to vary seasonally, and can be predicted using the temperature of the previous week (Smith & Dukes, 2018), a weekly to monthly acclimation time scale would be appropriate for LSMs to incorporate this process. It has also been shown that high growth temperature has a stronger negative impact on the instantaneous thermal response of $V_{cmax}$ than that of dark respiration (Smith & Dukes, 2017b). However, such effects occur above 30°C, whereas the maximum growth temperature of the sampled sites we used here is only ~28°C. More measures at hot sites would be helpful for future studies to understand acclimation behaviour over a larger range of temperatures.

Theory also predicts that the amount of active Rubisco should decline with temperature ($\beta_{qV} = -4.4% K^{-1}$), because the instantaneous response of $V_{cmax}$ to temperature is steeper than its acclimated response. At higher temperatures, less active Rubisco is required to achieve the value of $V_{cmax}$ indicated by the co-ordination hypothesis (Figure 1). Lower levels of Rubisco require lower levels of maintenance respiration for Rubisco turnover. Both predictions are quantitatively consistent with
observed negative responses of $R_{d,25}$ and $V_{cmax,25}$ to temperature (Table 1, Figure 2), although the goodness of fit to the data at 25°C is weaker than that at growth temperature.

The growth temperature-dependent trend in both $V_{cmax}$ and leaf $R_d$ emerges clearly from the data despite considerable scatter around the regression (Figure 2). Much of this scatter may be linked to within-site microclimatic variation (especially in PPFD) that is not accounted for in analyses of this kind. Consistent with this hypothesis, growth temperature explains a larger fraction of the variation in community-mean values of $V_{cmax}$ and $R_d$ (Table S1) than in individual species values. The diversity among species in other relevant traits and leaf life history may also explain some within-site variation in $V_{cmax}$ and $R_d$. For example, it has been shown that diverse hydraulic strategies can influence plant photosynthetic capacity under the same abiotic conditions (Zhu et al., 2018), while age-dependent leaf physiology can significantly influence $V_{cmax}$ and consequently the total canopy carbon uptake, e.g. observed in tropical evergreen forests (Albert et al., 2018). Optimality-based theory on respiration acclimation as presented here could (a) be applied globally at a community-mean level, and (b) potentially be refined by explicitly considering variations within the canopy in microclimate and differences among plant strategies and leaf life histories.

Equation (3) could potentially allow predictions of the responses of leaf $R_d$ to other environmental determinants, including vapour pressure deficit, elevation and CO$_2$. Our theory predicts a downregulation of $V_{cmax}$ and thus of $R_d$ (both at 25°C and $T_g$) as a response to increased atmospheric CO$_2$. Consequently, enhanced thermal acclimation in $V_{cmax}$ and $R_d$ ($\beta_{aV} = 2.7\% K^{-1}$ and $\beta_{aR} = 4.5\% K^{-1}$, $\beta_{qV} = \beta_{qR} = -5.4\% K^{-1}$) are expected at high CO$_2$. The data currently available do not allow us to test these predictions, for various reasons including (a) the limited environmental range covered by data, (b) correlations between potential explanatory variables and (c) uncertainties in the measurement methods used in manipulative experiments (Ainsworth & Long, 2005). Nevertheless, our theory provides testable predictions on the acclimation of $R_d$ to various environmental factors and also a simple, first-principles approach to directly predict the thermal acclimation of $R_d$, which is one of
the most important mechanisms missing from current LSMs (Huntingford et al., 2017).

4.3 | On the correlation between $R_d$ and $N_{\text{area}}$

Empirical relationships of both $V_{\text{cmax},25}$ and $R_{d,25}$ to area-based leaf nitrogen content ($N_{\text{area}}$) have been interpreted as showing ‘nitrogen limitation’ at the leaf level (Luo et al., 2004) and form the basis of $R_d$ prediction in some N-cycle enabled LSMs. However, recent studies have shown two problems with this interpretation. First, $V_{\text{cmax},25}$ accounts for only the metabolic component of $N_{\text{area}}$, whereas a large component of variation in $N_{\text{area}}$ is proportional to LMA (Dong et al., 2017b, Onoda et al., 2017). Here, we confirm that substantially more variation in $N_{\text{area}}$ can be explained by LMA than by $V_{\text{cmax}}$ or $R_d$. This finding suggests that $N_{\text{area}}$ is not the main determinant of either $V_{\text{cmax}}$ or $R_d$ (Table 2). Second, global patterns of variation in $V_{\text{cmax}}$ have been shown to be predictable from climate alone (Smith et al., 2019b), suggesting that $V_{\text{cmax},25}$ (and therefore $R_{d,25}$) is not determined by $N_{\text{area}}$, but rather primarily by photosynthetic potential – which is set by the local climatic environment. This potential in turn determines the metabolic component of $N_{\text{area}}$. Differences in soil N availability then primarily influence plant-level carbon allocation, instead of leaf-level N: the less soil N supply, the more carbon allocated belowground for N acquisition (LeBauer & Treseder, 2008, Poorter et al., 2012).

Our analysis therefore suggests an alternative to the common approach of carbon-nitrogen cycle coupling applied in LSMs, whereby leaf nitrogen is prescribed by plant functional types and used to predict $V_{\text{cmax}}$ and leaf $R_d$ at standard temperature, and enzyme kinetics determines their temperature response at both fast (half-hourly) and slower (weekly and longer) time scales. In our proposed approach, leaf nitrogen is determined jointly by LMA (which may differ among plant functional types) and $V_{\text{cmax}}$, $V_{\text{cmax}}$ and $R_d$ at standard temperature would then be considered independent of plant functional type, but allowed to acclimate gradually to environmental conditions following a simple optimality principle. Nitrogen availability would influence primarily the allocation of nitrogen among plant organs, i.e. roots versus leaves.
The theory developed here provides a first-principles approach to predicting the thermal acclimation of leaf $R_d$, a key process missing from current LSMs. According to both theory and data, the observed thermal acclimation of $R_d$ follows the optimization of $V_{\text{cmax}}$ as predicted by the coordination hypothesis. This acclimation dampens the instantaneous response of $R_d$ to temperature and shows little influence from other factors. The discrepancy between thermal acclimation and instantaneous thermal response implies that both $R_d$ and $V_{\text{cmax}}$, converted to 25$^\circ$C or any other arbitrarily chosen reference temperature, must decline with plant growth temperature. These principles are straightforward to incorporate in an LSM framework. The theory provides an explanation for observed correlations among $N_{\text{area}}$, $V_{\text{cmax}}$ and $R_d$ that differs from the common assumption that $N_{\text{area}}$ determines $V_{\text{cmax}}$ and $R_d$, and supports an alternative perspective on the coupling between terrestrial carbon and nitrogen cycles.

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Author contributions

H.W. and I.C.P. derived the theory and designed the study. H.W. carried out all the analyses and constructed the Figures and Tables, and wrote the first draft. All authors contributed to the interpretation of the results and to the text.

Data sharing and data accessibility

The GlopResp database is accessible at the TRY plant trait database (www.try-db.org) or by contacting Owen Atkin. The LCE dataset is publicly accessible at https://github.com/SmithEcophysLab/LCE/releases/tag/v1.1. The climate data and fractional absorbed photosynthetic active radiation data are publicly accessible from Climatic Research Unit (https://crudata.uea.ac.uk/cru/data/hrg/tmc/), and NASA’s OceanColor Web (https://oceancolor.gsfc.nasa.gov/data/seawifs/), respectively.

References


Smith NG, Keenan TF, Prentice CI et al. (2019a) Global photosynthetic capacity is optimized to the environment. Ecology letters, 22, 506-517.

Smith NG, Keenan TF, Prentice IC et al. (2019b) Global photosynthetic capacity is optimized to the environment. Ecology letters.


Table 1: Summary of Ordinary Least-Squares regressions for natural log-transformed leaf $R_d$ and $V_{cmax}$ and their ratio as a function of growth temperature. Both $R_d$ and $V_{cmax}$ have been converted to growth temperature ($R_{d,tg}$ and $V_{cmax,tg}$) and to 25°C ($R_{d,25}$ and $V_{cmax,25}$) from the measured leaf temperature, and normalized by site-mean leaf absorbed photosynthetic photon flux density. For comparison, the theoretical values of thermal sensitivities are shown together with the fitted coefficient and its confidence intervals. Non-significant coefficients are shown in grey.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Theoretical value</th>
<th>Fitted coefficient</th>
<th>Confidence intervals</th>
<th>Intercept (mean ± se)</th>
<th>$R^2$</th>
<th>df</th>
</tr>
</thead>
<tbody>
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<td>$R_{d,tg}$</td>
<td>0.037</td>
<td>0.033</td>
<td>0.029</td>
<td>0.038</td>
<td>9.335±0.051</td>
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<tr>
<td>$V_{cmax,tg}$</td>
<td>0.055</td>
<td>0.052</td>
<td>0.047</td>
<td>0.057</td>
<td>6.255±0.054</td>
<td>0.48</td>
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<td>$R_{d,tg}/V_{cmax,tg}$</td>
<td>−0.018</td>
<td>−0.017</td>
<td>−0.023</td>
<td>−0.011</td>
<td>3.044±0.061</td>
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<td>$R_{d,25}$</td>
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<td>−0.045</td>
<td>7.261±0.052</td>
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<tr>
<td>$V_{cmax,25}$</td>
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<td>−0.042</td>
<td>−0.047</td>
<td>−0.036</td>
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<td>$R_{d,25}/V_{cmax,25}$</td>
<td>0</td>
<td>−0.007</td>
<td>−0.012</td>
<td>0.001</td>
<td>3.302±0.060</td>
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</table>
Table 2: Summary statistics for Ordinary Least Squares regressions of leaf nitrogen content against leaf mass per area (LMA) and/or $R_d$ or $V_{c_{\text{max}}}$.

$R_d$ and $V_{c_{\text{max}}}$ are assessed at growth temperature ($R_{d_{tg}}$ and $V_{c_{\text{max}},tg}$), or 25°C ($R_{d,25}$ and $V_{c_{\text{max}},25}$). The fitted slopes are shown together with the intercept (mean ± standard error), the adjusted coefficient of determination ($R^2$) and the degrees of freedom (df). All variables were natural-log transformed.

<table>
<thead>
<tr>
<th>$V_{c_{\text{max}},tg}$</th>
<th>$V_{c_{\text{max}},25}$</th>
<th>$R_{d_{tg}}$</th>
<th>$R_{d,25}$</th>
<th>LMA</th>
<th>Intercept</th>
<th>$R^2$</th>
<th>df</th>
</tr>
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<tr>
<td>0.083±0.019</td>
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<td></td>
<td>0.409±0.059</td>
<td>0.02</td>
<td>935</td>
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<tr>
<td>0.058±0.015</td>
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<td>0.491±0.021</td>
<td>-1.849±0.107</td>
<td>0.39</td>
<td>934</td>
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<td></td>
<td></td>
<td>-0.199±0.081</td>
<td>0.12</td>
<td>935</td>
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Figure Legend

Figure 1: Schematic of the thermal sensitivities of leaf maximum carboxylation capacity ($V_{\text{cmax}}$) and dark respiration ($R_d$). Step 1: The “co-ordination hypothesis” predicts a positive response of $V_{\text{cmax}}$ to growth temperature ($T_g$). Due to increasing Rubisco oxygenation relative to carboxylation, a higher $V_{\text{cmax}}$ is required to achieve the optimal photosynthetic rate at higher growth temperatures ($T'_g$). Step 2: When temperature increases, the value of $V_{\text{cmax}}$ achieved through the instantaneous response of Rubisco is super-optimal. Consequently, the amount of Rubisco (indexed by $V_{\text{cmax}}$ at the standard reference temperature of 25°C) must be “down-regulated” from $V_{\text{cmax,25}}$ to $V'_{\text{cmax,25}}$. Step 3: We hypothesize that respiratory and photosynthetic capacities are linked such that leaf $R'_{\text{d,25}}$ at growth temperature is a fixed fraction of $V'_{\text{cmax,25}}$. Step 4: Just as for $V_{\text{cmax}}$, leaf $R'_{\text{d,25}}$ at the new growth temperature is lower than $R_{\text{d,25}}$ at the original growth temperature, implying an acclimated/adapted thermal response that is less steep than the instantaneous response.

Figure 2: Natural log-transformed leaf dark respiration ($R_d$), maximum carboxylation capacity ($V_{\text{cmax}}$) and their ratio as a function of growth temperature (mGDD$_0$). Both $R_d$ and $V_{\text{cmax}}$ are standardized to growth temperature and to 25°C, and normalized by site-mean leaf absorbed photosynthetic photon flux density. Solid blue lines are the fitted lines from Ordinary Least Squares regressions. Solid black lines are theoretical predictions. Dashed lines represent instantaneous temperature responses.