Thermal acclimation of leaf respiration consistent with optimal plant function

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Introduction

Terrestrial plant respiration is a major component of the global carbon cycle, releasing ca. 60 Pg C yr⁻¹ to the atmosphere: six times more than anthropogenic CO₂ emissions from all sources combined (Ciais et al. 2014). About half this flux is leaf maintenance respiration in darkness ($R_d$) (Atkin et al. 2007). $R_d$ is highly temperature-dependent, following a near-exponential relationship over short time-scales (Heskel et al. 2016).

It has been predicted that global warming will increase $R_d$ and accelerate future climate change via a carbon-climate feedback (Cox et al. 2000; Huntingford et al. 2013). However, large uncertainties in future carbon cycle responses to warming persist, likely because model formulations of the temperature responses of photosynthesis and $R_d$ remain poorly constrained by both theory and observations (Ziehn et al. 2011; Booth et al. 2012; Friedlingstein et al. 2014).

Representations of $R_d$ in most land surface models (LSMs) are based on the instantaneous temperature response of $R_d$, governed by enzyme kinetics, relative to a baseline rate, typically at 25°C ($R_{d,25}$) (Atkin et al. 2017). $R_{d,25}$ is commonly assumed to be proportional either to area-based leaf nitrogen content ($N_{area}$), or to the maximum rate of carboxylation at 25°C ($V_{cmax,25}$) (Rogers 2014). Predicting photosynthetic traits from $N_{area}$ is problematic because a large fraction of leaf N is contained in cell walls (Onoda et al. 2017), and significant fractions are also allocated to other non-photosynthetic functions including defense, storage and osmoregulation (Dong et al., 2017). Many models treat $V_{cmax,25}$ and $R_{d,25}$ as constant for each plant functional type (PFT), but spatial and temporal trait variations within PFTs are to be expected (Wang et al. 2017b) and have been reported (Kattge et al. 2011, Atkin et al. 2015). Moreover, trait differences observed among PFTs could be caused by acclimation to different environments, rather than intrinsic properties of PFTs.

Experiments have shown acclimation of $R_d$, such that its response to growth temperature over a week or longer is shallower than its response to temperature variation in the short term (Atkin & Tjoelker 2003; Gifford 2003; Smith & Dukes 2013; Aspinwall et al. 2016; Drake et al. 2016; Reich et al. 2016; Scafaro et al. 2017). The acclimation of $R_d$ to growth temperature is also evident in spatial observations.
Atkin et al. 2015; Slot & Kitajima 2015), showing a far weaker pole-to-equator gradient than would be expected if field-measured $R_d$ followed the instantaneous response of $R_d$ to temperature. By analysing the temperature responses of $R_d$ in different datasets, Vanderwel et al. (2015) demonstrated consistency between the observed spatial pattern of $R_d$ and the acclimation of $R_d$ over time. Similar levels of thermal acclimation of photosynthesis and respiration have been shown to occur in different PFTs (Campbell et al. 2007; Smith & Dukes 2017b). Pervasive $R_d$ acclimation implies a weaker positive carbon-climate feedback than implied by the temperature response of enzyme kinetics (i.e. the instantaneous response) (Reich et al. 2016; Smith et al. 2016; Huntingford et al. 2017). Neglecting acclimation in LSMS is thus a potential major source of bias in Earth system model predictions (Smith & Dukes, 2013), as recently demonstrated by Huntingford et al. (2017).

Still missing is a theoretical explanation for $R_d$ acclimation. Conclusions from empirical studies alone (Wright et al. 2006) remain subject to the limitations of sampled region, observational period and experimental design. A theoretical basis is essential to build confidence in carbon-cycle predictions (Prentice et al., 2015). As advocated e.g. by Marquet et al. (2014), theories grounded in first principles have the potential to generate explicit quantitative predictions with few assumptions or unconstrained parameters, providing independent standards for comparison with empirical data. Here, we develop such a theory for $R_d$ acclimation, based on the assumption that the various metabolic functions of $R_d$ are coordinated with photosynthetic capacity, indexed by $V_{cmax}$. In combination with predictions of optimally acclimated $V_{cmax}$ based on the coordination hypothesis (Chen et al., 1993; Haxeltine & Prentice, 1996; Maire et al., 2012; Togashi et al., 2018) – whereby optimal $V_{cmax}$ is just sufficient to use available resources under current average environmental conditions – our approach allows us to formulate and test the sensitivities of the traits of interest ($R_d$, $V_{cmax}$) to temperature, yielding results applicable to all plants.

Our theory is based on the concept of eco-evolutionary optimality, which derives from the premise that natural selection favours efficient resource allocation by eliminating unsuccessful or uncompetitive trait combinations (Givnish 1986a; Tilman 1999). Optimality-based theories have proven predictive power for plant functions...
including leaf venation networks (Blonder et al. 2017), stomatal behaviour (Cowan 1986; Givnish 1986b; Farquhar et al. 2002; Lin et al. 2015; Wolf et al. 2016; Dewar et al. 2018), leaf-level CO₂ drawdown (Prentice et al. 2014), phenology (Kikuzawa et al. 2013; Xu et al. 2017), leaf nitrogen content (Wright et al. 2003; Maire et al. 2012; Dong et al. 2017) and adaptations to elevation (Wang et al. 2017a); and ecosystem processes including vegetation succession (Weng et al. 2017) and primary production (Keenan et al. 2016; Wang et al. 2017b). By linking Rd with optimal Vcmax acclimation (Box 1), we derive quantitative predictions of the thermal sensitivities of acclimated Rd and Vcmax evaluated at the prevailing growth temperature, and at 25°C. Our theory implies that correlations between Vcmax,25 and Narea, and between Rd,25 and Narea primarily reflect the N requirements of metabolism (as implied by the coordination hypothesis) rather than ‘N limitation’ of either Vmax or Rd – that is, the amount of ‘metabolic’ N in the leaf is optimized for current conditions. These predictions are tested using two extensive field observational datasets (Atkin et al., 2015; Smith & Dukes, 2017).

Materials and Methods

Theoretical framework

Leaf dark respiration (Rd) is closely coupled with photosynthetic activity (Hoefnagel et al. 1998; Wright et al. 2004; Noguchi & Yoshida 2008; Tcherkez 2012). As described by the standard biochemical model of photosynthesis (Farquhar et al. 1980), the instantaneous rate of photosynthesis by C3 plants is limited either by the capacity of the enzyme Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) for the carboxylation of RuBP (Vcmax), or by the rate of electron transport for the regeneration of RuBP, which depends on absorbed light and the electron transport capacity (Jmax). Rd is used to support diverse 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 Rd variation and is expected to scale closely with Vcmax, which sets the daily maximum photosynthetic rate achieved by leaves under natural growing conditions (Amthor 2000; Atkin et al. 2000; Cannell & Thornley 2000; Bouma 2005).
We start from the assumption that at the prevailing growth temperature, \( V_{\text{cmax}} \) is proportional to the overall metabolic activity of the leaf, which is supported by \( R_d \).

The dimensionless factor \( b \) (main text equation (1)) is expected to be constant (for all plants) at a given growth temperature. The value of \( b \) at 25°C (\( b_{25} \)) has been given as 0.011 (Farquhar et al., 1980) or 0.015 (Collatz et al., 1991). A constant \( b_{25} \) implies that a certain quantity of respiratory enzymes is required for the maintenance of a certain quantity of Rubisco. Our initial attention therefore focuses on the prediction of optimal \( V_{\text{cmax}} \) achieved by the acclimation of photosynthetic processes.

We hypothesize that \( V_{\text{cmax}} \) of leaves at any canopy level acclimates to the current environment so as to be just sufficient to allow exploitation of the average available light. This is the “strong form” (Togashi et al., 2018) of the coordination hypothesis (Chen et al., 1993; Haxeltine & Prentice, 1996; Maire et al., 2012) (contrasted with the “weak form” that assumes that the total metabolic N content of the leaf is prescribed, so only the allocation of N to carboxylation versus electron transport capacities is optimized). The hypothesis leads to a prediction of the ecophysiologically relevant (acclimated) values of both \( R_d \) and \( V_{\text{cmax}} \) at the prevailing temperature, i.e. the average growth temperature (\( T_g \)). Values of \( R_d \) and \( V_{\text{cmax}} \) at 25°C (\( R_{d,25} \) and \( V_{\text{cmax},25} \)) are related to quantities of enzymes, while values at growth temperature (\( R_{d,tg} \) and \( V_{\text{cmax},tg} \)) are hypothesized to be optimized by the plant.

Numerical conversion between values applying to different temperatures is accomplished by applying functions (Kattge & Knorr, 2007; Heskel et al., 2016) that describe how the instantaneous rates increase with temperature due to enzyme kinetics. To achieve the same level of \( V_{\text{cmax}} \), a smaller quantity of Rubisco (and therefore a smaller \( V_{\text{cmax},25} \)) is required at a higher temperature (Fig. 1). The coordination hypothesis predicts acclimated values of \( R_{d,tg} \) and \( V_{\text{cmax},tg} \) that increase with growth temperature, but less steeply than the responses of enzyme kinetics (Togashi et al., 2018). As a result, acclimated values of \( R_{d,25} \) and \( V_{\text{cmax},25} \) are predicted to decline with growth temperature. Moreover, because the instantaneous responses of \( R_d \) and \( V_{\text{cmax}} \) to temperature are slightly different, these differences have to be compensated by differences in the acclimated responses of \( R_{d,tg} \) and \( V_{\text{cmax},tg} \).

The reasoning set out above can be represented mathematically, leading to a theoretical derivation of the thermal sensitivities of \( R_d, V_{\text{cmax}} \) and their ratio (assessed...
at both $T_a$ and $25^\circ C$) as follows. We first use the coordination hypothesis to predict the fractional sensitivity of $V_{cmax,lg}$ to growth temperature (denoted $\beta_{dV}$, the subscripts $a$ and $i$ here represent the acclimated and instantaneous responses, respectively), which is predicted to be less than the instantaneous response, i.e. $\beta_{dV} < \beta_{iV}$. The hypothesized proportionality between $R_d$ and $V_{cmax}$ then links the thermal sensitivity of $R_d$ ($\beta_{AR}$) with $V_{cmax,lg}$, since $\beta_{AR} = \beta_{b} + \beta_{dV}$. Here $\beta_{b}$ quantifies the variation of $b$ with temperature, which is due to the slight lower instantaneous thermal responses of $R_d$ ($\beta_{IR}$) than $V_{cmax}$, i.e. $\beta_{b} = \beta_{IR} - \beta_{iV} < 0$. Making use of the general properties of logarithms, $\beta_{aV25}$ and $\beta_{dR25}$ are predicted as a secondary consequence of $V_{cmax,lg}$ and $R_{d,lg}$ acclimation combined with enzyme kinetics: $\beta_{aV25} = \beta_{aV} - \beta_{iV} = \beta_{dR25} = \beta_{dR} - \beta_{IR} < 0$.

**Quantitative predictions**

Our approach (Box 1) is based on the assumption that acclimated $R_d$ is proportional to photosynthetic capacity, represented by $V_{cmax}$, where their ratio ($b$) may be a function of temperature:

$$R_d = bV_{cmax}$$  \hspace{1cm} (1)

We therefore first explain quantitative predictions of the thermal acclimation of optimal $V_{cmax}$.

The coordination hypothesis predicts a spatial and temporal pattern in $V_{cmax}$ assessed at growth temperature, reflecting acclimation to the prevailing environmental conditions of an individual leaf. In response to environmental variations, it predicts that $V_{cmax}$ can vary vertically within the canopy, geographically among sites, and temporally with atmospheric CO$_2$ concentration and climate (Haxeltine & Prentice 1996; Ainsworth & Long 2005; Maire et al. 2012). $V_{cmax}$ values have been shown experimentally to acclimate to sustained changes in growth temperature, such that $V_{cmax}$ assessed at growth temperature (hereafter $V_{cmax,lg}$) increases with growth temperature, while $V_{cmax,25}$ declines, along with the Rubisco amount and the fraction of leaf N allocated to Rubisco (Scafaro et al. 2017).

Quantitative predictions of $V_{cmax}$ can be obtained from the co-ordination hypothesis by equating the Rubisco-limited ($A_C$) and electron transport-limited ($A_I$) rates of C$_3$
photosynthesis in the Farquhar et al. (1980) model (Wang et al., 2017b). For
simplicity, we shall assume that the light response of $A_I$ under natural light conditions
is effectively linear up to the point at which $A_C$ becomes limiting, implying that
limitation of photosynthesis by $J_{\text{max}}$ under average field conditions is generally
avoided (Wang et al. 2014; Keenan et al. 2016; Dong et al. 2017; Wang et al. 2017a;
Togashi et al. 2018). Thus, under field conditions the coordination hypothesis predicts
(Dong et al., 2017; Togashi et al., 2018):

$$V_{c_{\text{max,fg}}} \approx \varphi_0 I_{\text{abs}} (\chi c_a + K) / (\chi c_a + 2I^*)$$  (2)

where $\varphi_0$ is the intrinsic quantum efficiency of photosynthesis, which is independent
of temperature over the normal range of metabolic activity (Collatz et al. 1990), and
$I_{\text{abs}}$ is the leaf absorbed photosynthetic photon flux density (PPFD). $\chi$ is the ratio of
leaf-internal to ambient partial pressure of CO$_2$, $c_a$ is the ambient partial pressure of
CO$_2$, $I^*$ is the photorespiratory compensation point, and $K$ is the effective Michaelis-
Menten coefficient of Rubisco. $I^*$ and $K$ are temperature-dependent following
Arrhenius relationships measured e.g. by Bernacchi et al. (2001). The least-cost
hypothesis (Prentice et al., 2014; Wang et al., 2017b) predicts optimal $\chi$ as a function
of growing-season mean values of temperature ($T_g$) and vapour pressure deficit ($D$),
and elevation ($z$). This prediction is quantitatively supported by worldwide
measurements of $\chi$ across species and environments (Wang et al. 2017b). Equation (2)
yields estimates of $V_{c_{\text{max,fg}}}$ given $\chi$ and field-relevant average values of $c_a$, temperature
and PPFD. The theoretical temperature dependence of $V_{c_{\text{max,fg}}}$ arises from the separate
temperature responses of $\chi$, $I^*$ and $K$. The sensitivity of $V_{c_{\text{max,fg}}}$ to temperature can be
obtained by differentiating equation (2). Evaluating the result under standard
conditions ($T_g = 25^\circ C$, $D = 1$ kPa, $z = 0$, $c_a = 40$ Pa) yields $\beta_{I^* V} = 5.5\% K^{-1}$ ($\beta_{I^* V}$ is the
fractional sensitivity of $V_{c_{\text{max,fg}}}$ to temperature after acclimation: see Box 1 for
definitions, Figure 1 for a graphical explanation, and Appendix 1 for derivations).
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 on the sensitivity of $\chi$ to temperature (0.9% K$^{-1}$).
$V_{c_{\text{max,fg}}}$ is then corrected from $T_g$ to 25$^\circ$C using the enzyme-kinetic temperature
response of $V_{c_{\text{max}}}$ (Kattge & Knorr 2007). Evaluated at 20°C (mean $T_g$ in our dataset),
this function yields an instantaneous thermal sensitivity of $V_{c_{\text{max}}}$ of $\beta_{I^* V} = 9.9\% K^{-1}$,
higher than the acclimated thermal sensitivity \( \beta_{aV} = 5.5\% \text{ K}^{-1} \). The thermal sensitivity of acclimated \( V_{cmax,25} \) is predicted as the difference between the thermal sensitivities of \( V_{cmax} \) acclimated to the growth temperature \( (V_{cmax,tg}) \) and the instantaneous enzyme-kinetic response of \( V_{cmax} \) (Box 1: \( \beta_{aV25} = \beta_{aV} - \beta_{iV} = -4.4\% \text{ K}^{-1} \)).

Heskel et al. (2016) provided an estimate of \( R_d \) at a reference temperature \( (T_{ref}) \):

\[
\ln R_{d,ref} = a + 0.1012T_{ref} - 0.0005T_{ref}^2
\]

where \( a \) is an empirical constant varying among biomes, representing the natural log of the value of \( R_d \) at 0°C. The enzyme-kinetic response of \( R_d \) to temperature \( (\beta_{iR}) \) as given by Heskel et al. (2016) is \( 8.1\% \text{ K}^{-1} \) at the mean \( T_g \) of the data. The enzyme-kinetic thermal response of \( R_d \) is slightly smaller than \( \beta_{iV} \), and leads to a thermal response of parameter \( b \) in equation (1) given by the difference between \( \beta_{iR} \) and \( \beta_{iV} \) \( (\beta_b = \beta_{iR} - \beta_{iV} = -1.8\% \text{ K}^{-1} \)). This then generates a prediction of \( \beta_{ad} = 3.7\% \text{ K}^{-1} \) and \( \beta_{adr25} = -4.4\% \), which is the same as \( \beta_{aV25} \), consistent with the assumption that \( b_{25} \) is a constant (Box 1). Derivations are provided in Supporting Information (Appendix 1).

**Empirical analyses**

**Photosynthesis and respiration data**

We combined two \( R_d \) datasets. GlobResp (Atkin et al. 2015) contains measurements of \( R_d \), \( V_{cmax} \), \( N_{area} \) 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 (including \( R_d \) and \( V_{cmax} \)) and leaf chemical traits (including \( N_{area} \) and LMA) from 98 species at 12 locations spanning 53° latitude in North and Central America (Fig. S1). \( R_d \) measurements in both datasets followed the same protocol. Both were taken on fully expanded leaves in daytime after a period of dark adjustment. Inhibition of respiration in the light was not assessed. \( V_{cmax} \) values in GlobResp were estimated by the ‘one-point method’ whereas those in LCE were estimated from full \( A-c_i \) curves; these methods give closely similar results (De Kauwe et al. 2016). Replicated measurements in LCE on the same species and site were averaged. Juvenile samples were excluded. We index \( T_g \) by the mean temperature
during the thermal growing season when temperatures are above 0°C, mGDD0 (Harrison et al. 2010). $V_{cmax}$ and $R_d$ values in both datasets are provided with information about measurement temperatures. The values were adjusted both to mGDD0 and to 25°C using the relevant kinetic responses, as given by (Kattge & Knorr 2007) and Heskel et al. (2016). 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 mGDD0 for each location. Thermal acclimation of $R_d$ should apply to both C3 and C4 plants, but our theoretical prediction of $V_{cmax}$ acclimation here is developed for C3 plants, and we did not include C4 species in our analysis.

**Statistical analysis**

To test our predictions of the acclimated thermal sensitivities of $R_d$ and $V_{cmax}$ quantitatively, the $R_d$ and $V_{cmax}$ data (assessed at mGDD0 and 25°C) were first normalized with site-mean PPFD absorbed by leaves (PPFD$_L$) before performing the Ordinary Least Squares (OLS) regression against temperature. This normalization is appropriate because $V_{cmax}$ is both predicted (see Appendix 1) and observed to vary in proportion to PPFD (Niinemets & Keenan, 2012). If it were omitted, the positive effect of PPFD on $R_d$ and $V_{cmax}$ would contribute to the fitted slope of mGDD0 due to the strong correlation between those two variables (Fig. S2). Site-mean PPFD$_L$ was estimated from growing-season total incident PPFD at the top of the canopy (PPFD$_0$):

$$PPFD_L \approx fPPFD_0/L$$  \hspace{1cm} (4)

where $f$ is the fraction of incident PPFD absorbed by the canopy (from SeaWiFS data: (Gobron et al. 2006; Kelley et al. 2013) and $L$ is the leaf area index estimated from Beer’s law:

$$L \approx -(1/k) \ln (1-f)$$  \hspace{1cm} (5)

with $k = 0.5$ (Dong et al. 2017). This general approximation is used because we do not have information on the light levels of species occupying different canopy strata. PPFD$_0$ was calculated from CRU CL2.0 data using SPLASH (Davis et al. 2017). We applied OLS linear regression of normalized (and natural log-transformed) $R_d$ and $V_{cmax}$ values against mGDD0. The resulting slope coefficients are directly comparable
with the thermal sensitivities predicted by theory. To check the impact of the PPFD normalization, we also performed regressions without it. Thermal acclimation of \( R_d \) and \( V_{\text{cmax}} \) was further tested within PFTs, with species assigned to deciduous and evergreen needleleaf and broadleaf trees, deciduous and evergreen shrubs, and C3 herbaceous plants.

To test the hypothesis that \( R_d \) is mainly determined by \( V_{\text{cmax}} \) rather than leaf or soil characteristics, we also included LMA and soil pH as additional predictors in the OLS regression above. LMA carries information on the structural component of plant leaves. Broadly speaking, higher soil pH indicates higher soil fertility (Jenny, 1994; Sinsabaugh & Follstad Shah, 2012), and pH has been shown to influence \( \chi \) (Wang et al. 2017b). These two covariates were selected to test any potential influences of leaf structure and soil nutrient availability, respectively, on \( R_d \). An estimate of soil pH for each location was extracted from the Harmonized World Soil Database (http://www.iiasa.ac.at). We applied Ordinary Least Squares (OLS) linear regression of \( R_d \) versus \( V_{\text{cmax}} \) (standardized to 25°C and to mGDD0, without transformation) to estimate \( b_{25} \) and \( b \) directly from the fitted slopes. Relationships of \( N_{\text{area}} \) with \( V_{\text{cmax}}, R_d, \) and LMA were also tested by OLS multiple linear regression.

**Results**

**Sensitivity of acclimated \( R_d \) and \( V_{\text{cmax}} \) to growth temperature**

The predicted sensitivity of \( V_{\text{cmax}} \) to growth temperature (\( \beta_{\text{av}} \)) was 5.5% K\(^{-1} \) under standard environmental conditions. This value is identical with the fitted regression coefficient of normalized and transformed \( V_{\text{cmax},tg} \) against mGDD0 (5.6% ± 0.3%; mean and 95% confidence interval, \( R^2 = 0.50 \)) (Table 1, Fig. 2).

The prediction that \( b \) should decline with temperature by 1.8% K\(^{-1} \) was consistent with the fitted regressions of the ratio of \( R_{d,tg} \) to \( V_{\text{cmax},tg} \); 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 mGDD0, as predicted (Table 1, Fig. 2). The fitted temperature response of \( R_{d,tg} \) was consistently about 2% less steep than that of \( V_{\text{cmax},tg} \) (Table 1).
The canonical value of $b_{25} = 0.015$, as assumed 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 $V_{cmax,25}$ (Table 2). The regression lines of $R_{d,lg}$ with respect to $V_{cmax,lg}$ when fitted to data in low ($T_g < 15\,\text{C}$), medium ($15\,\text{C} < T_g < 25\,\text{C}$) and high ($T_g > 25\,\text{C}$) temperature classes separately (Fig. S3) became shallower toward higher temperature classes, also consistent with the prediction of a negative response of $b$ to temperature.

The difference between the enzyme-kinetic sensitivities of $R_d$ and $V_{cmax}$ to temperature implies that the sensitivity of acclimated $R_{d,lg}$ to temperature ($\beta_{aR}$) is 1.8\% lower than that of $V_{cmax,lg}$ ($\beta_{aV}$), implying a theoretical optimum rate of increase of $R_{d,lg}$ by 3.7\% per degree. These theoretical responses are very close to those seen in the observations, but rather more shallow than the 9.9\% and 8.1\% K$^{-1}$ predicted for the short-term responses of $V_{cmax}$ and $R_d$ from enzyme kinetics (Fig. 1). 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 identical (−4.4 ± 0.3\% K$^{-1}$) to our theoretical prediction; the observed response of $R_d^{25}$ (−4.9 ± 0.3\% K$^{-1}$) is marginally larger than the prediction.

Regressions performed without PPFD-normalization (Table S1) 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 – as expected due to the confounding of PPFD and temperature effects, 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 11-16\% for $V_{cmax}$ and $R_d$ at 25°C.

Thermal acclimation of $V_{cmax}$ within PFTs was shown to be broadly consistent with the universal relationships evident in the whole dataset (Table S2). Consistent with Campbell et al. (2007) and Smith and Dukes (2017b), no significant differences in thermal acclimation were found between PFTs with different leaf phenology (evergreen versus deciduous), leaf form (needleleaf versus broadleaf), or life form (trees versus shrubs versus herbaceous plants), although the fitted slope for evergreen
broadleaf trees is higher than predicted. Thermal acclimation of $R_d$ within PFTs shows more variable results. Evergreen broadleaf trees show higher than predicted thermal sensitivities of both $R_d$ and $V_{cmax}$, but the fitted slope of their ratio versus growth temperature ($-1.8\% \pm 0.4\%$) is identical with our predicted value ($-1.8\%$).

Microclimatic acclimation (Niinemets & Keenan 2012), the likelihood that many measured leaves were at least partially shaded (Keenan & Niinemets 2017), and genetic variations involving different plant strategies may all have contributed to the within-site variations in $R_d$ and $V_{cmax}$ reflected in the vertical scatter of points in Fig. 2. The theory was applied here to predict variations in $R_d$ and $V_{cmax}$ across sites, however, and as much as 45% and 60% variation in the community-mean $R_{d,tg}$ and $V_{cmax,tg}$ respectively could indeed be explained by growth temperature – with responses in quantitative agreement with predictions (Table S3).

*Relationships between dark respiration and carboxylation capacity*

We examined the relationships between $R_d$, $V_{cmax}$ and other factors, in order to test our hypothesis that $R_d$ is principally determined by $V_{cmax}$. We found that measured $R_d$ and $V_{cmax}$ 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 2).

*Relationships of dark respiration and photosynthetic capacity to other variables*

Relationships of $R_d$ and $V_{cmax}$ to $N_{area}$ were similar in strength when normalized to 25˚C ($R^2 = 0.14$ and 0.12) (Table 3), but notably weaker when considered at growth temperature ($R^2 = 0.05$ for $R_{d,tg}$ and 0.02 for $V_{cmax,tg}$). LMA and $V_{cmax,25}$ together accounted for 42% variation in $N_{area}$, but most of this explanatory power coming from LMA (Table 3). Similarly, LMA and $R_{d,25}$ together explained 41% variation in $N_{area}$, but again most of this explanatory power is due to LMA (Table 3).

Considered on their own, neither LMA nor soil pH provided explanatory power in the variations of $R_d$ and $V_{cmax}$ (whether at 25˚C and growth temperature). The inclusion of one or the other in addition to mGDD$_0$ as a predictor provided negligible increases in explained variance (Table S4).
Discussion

Acclimation of leaf dark respiration follows the acclimation of carboxylation capacity

Farquhar et al. (1980) modelled instantaneous $R_d$ at 25°C as a fixed fraction (1.1%) of $V_{cmax}$ at 25°C. Collatz et al. (1991) – citing Farquhar et al. (1980) – modelled $R_d$ at 25°C as 1.5% of $V_{cmax}$ at 25°C. Our results show that $R_d$ and $V_{cmax}$ are indeed closely related, whether standardized to growth temperature or to 25°C, albeit with substantial scatter around the relationship. Moreover, the coefficient relating $R_{d,25}$ to $V_{cmax,25}$ estimated from this large global dataset ($b_{25} = 0.014 \pm 0.01$, Table 2) is indistinguishable from the value of 0.015 used by Collatz et al. (1991). We also predict a slight negative response of $b$ to temperature due to the difference in the kinetic responses of $R_d$ and $V_{cmax}$. This expectation is consistent with other studies (De Kauwe et al. 2016), and well supported by the finding here of a temperature effect in the regression of $b$ (estimated from the $R_{d,tg}$ to $V_{cmax, tg}$ ratio), but not in that of $b_{25}$ (estimated from the $R_{d,25}$ to $V_{cmax,25}$ ratio). The former provides a value of $\beta_b$ close to our theoretical value of 1.8% K$^{-1}$ (Table 1).

Atkin et al. (2015) indicated a decline in $b_{25}$ with increasing growth temperature. Their dataset that did not included the LCE data. To compare our analysis with that of Atkin et al. (2015), we assessed the response of $b_{25}$ to mGDD$_0$ after excluding the LCE data. The response of $b_{25}$ to mGDD$_0$ became significant ($p = 0.0076$) but its sensitivity ($\beta_{b_{25}} = -0.009 \pm 0.003$) was much lower than that of $b$ ($\beta_b = -0.024 \pm 0.003$, $p < 0.001$). Atkin et al. (2015) considered the mean temperature of the warmest three-month period as growth temperature (TWQ), whereas here all days with temperature above 0°C are accounted in the growing season – thus growth temperature by our definition (mGDD$_0$) is lower than TWQ, except for the low and high ends of the scale (Fig. S4). This methodological difference might have contributed to a slightly different conclusion regarding the temperature sensitivity of $b_{25}$.

Equation (1) would potentially allow predictions of the responses of $R_d$ to other environmental determinants, including vapour pressure deficit, elevation and CO$_2$, if these too are determined by the environmental responses of $V_{cmax}$. However, the data currently available do not allow us to test these predictions, due to either the limited environmental range covered by the data (such as elevation or CO$_2$) or the strong...
correlations between potential explanatory variables (including light and temperature, Fig. S2). Nevertheless, our theory provides a simple, first-principles approach to predicting the thermal acclimation of $R_d$ – one of the most important mechanisms missing from current LSMs (Huntingford et al. 2017).

**Observed temperature responses are consistent with optimal plant function**

Many ecosystem models assume that $R_d$ and $V_{cmax}$ respond to temperature following the same functions that are routinely observed in short-term studies, disregarding acclimation. Our results contradict this assumption. Optimally acclimated values of both fluxes do increase with temperature, but much less steeply than expected from short-term responses. Instead it appears that leaves ‘discount’ enzyme-kinetic responses, so the two limiting photosynthetic rates remain similar under the prevailing growth temperature. Positive temperature responses arise because of the differential temperature sensitivities of two key quantities – the effective Michaelis-Menten coefficient of Rubisco ($K$) and the photorespiratory CO$_2$ compensation point ($\Gamma^*$) – in the Farquhar et al. (1980) model. These predictions are supported by the finding of temperature responses in the datasets evaluated here: that is, after the $R_d$ and $V_{cmax}$ values have been corrected to site-specific mean growing-season temperatures, we find responses closely similar to the predicted optimal responses (Fig. 2).

**The acclimated sensitivity of dark respiration to warming**

Acclimated $R_d$ is predicted to increase with temperature by 3.7% per degree. This long-term (weekly or longer) sensitivity is supported by the data and is smaller than the enzyme-kinetic sensitivity of either $R_d$ or $V_{cmax}$. This response is generally conservative among PFTs.

Reich et al. (2016) conducted outdoor open-air warming experiments on ten boreal and temperate tree species. Whole plants were warmed by 3.4˚C during four growing seasons, and showed even stronger acclimation of $R_d$ and an acclimated sensitivity of only 1.5% per degree. Slot and Kitajima (2015) indicated a value of 5.7% for the temperature sensitivity of $R_d$ based on a meta-analysis of 43 independent experiments (mostly short-term, laboratory studies). Our theory predicts $\beta_{air}$ as 3.7% per degree warming, intermediate between the values found by Reich et al. (2016) and Slot and
In a study that acclimated plants for seven days, Smith and Dukes (2017b) found even less acclimation of $R_d$ than either of those studies. The differences among these three studies suggest that the time scale of exposure to elevated temperatures might play a role.

Heskel et al. (2016) presented data from different biomes that can be compared to our predictions of the acclimation of $R_d$. We can estimate $a$ in equation (3) by rearrangement, with $T_{\text{ref}} = 25^\circ$C:

$$a = \ln R_{d,25} - 0.1012 \times 25 - 0.0005 \times 25^2$$

$$= \ln b_{25} + \ln V_{\text{cmax},25} - 0.1012 \times 25 - 0.0005 \times 25^2$$

(6)

The thermal sensitivity of $a$ should therefore be the same as that of $\beta_{aR25}$. Its decline from tundra to tropical forests (Heskel et al., 2016) is the result of $R_d$ acclimation to growth temperature (Slot & Kitajima 2015; Vanderwel et al. 2015). Our theory estimates $a$ independently: $a = -2.502$ when $V_{\text{cmax},25} = 50$ µmol m$^{-2}$ s$^{-1}$ (i.e. equivalent to $R_{d,25} = 1.4$ µmol m$^{-2}$ s$^{-1}$), or $a = -1.407$ when $V_{\text{cmax},25} = 150$ µmol m$^{-2}$ s$^{-1}$ (i.e. equivalent to $R_{d,25} = 4.2$ µmol m$^{-2}$ s$^{-1}$). These values are similar to those derived from observations by Heskel et al. (2016): lower for warm biomes (e.g. $a = -2.749$ for lowland tropical rainforest), where we would expect relatively low $V_{\text{cmax},25}$; and higher for cold biomes (e.g. $a = -1.604$ for tundra), where we would expect relatively high $V_{\text{cmax},25}$. These values from 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 – very close to our theoretical prediction of $\beta_{aR25} = -4.4\%$ K$^{-1}$.

Relationships of respiration and photosynthetic capacity with leaf N

It is often assumed that $R_d$ and $V_{\text{cmax}}$ (assessed at standard temperature) are closely related to $N_{\text{area}}$, and empirical studies have reported relationships of both quantities to $N_{\text{area}}$ (Reich et al. 1998; Meir et al. 2001; Wright et al. 2004; Atkin et al. 2015). Correlation of both $R_{d,25}$ and $V_{\text{cmax},25}$ with $N_{\text{area}}$ is expected because of the significant fraction of $N_{\text{area}}$ that is contained in Rubisco and other photosynthetic proteins, and the functional relationship between $R_d$ and $V_{\text{cmax}}$. ($N_{\text{area}}$ should always be compared to $R_d$ and $V_{\text{cmax}}$ corrected to a common temperature, because the amount of “metabolic”
N required to achieve a given catalytic activity is strongly dependent on temperature.) We found the expected positive relationships of $R_{d,25}$ and $V_{cmax,25}$ to $N_{area}$, but they were not strong ($R^2 = 0.11$ and 0.14, Table 3). Onoda et al. (2017) noted the substantial fraction of leaf N allocated to cell walls, in addition to the fraction contained in photosynthetic proteins. Our finding that far more variation in $N_{area}$ can be explained by LMA than by $V_{cmax}$ (Table 3) suggests that the structural (cell wall) component of $N_{area}$ is important (see also Dong et al. 2017). $N_{area}$ is thus not a straightforward predictor of either $V_{cmax}$ or $R_d$, but rather contains both a metabolic component (related to $V_{cmax,25}$) and a structural component proportional to LMA (Dong et al., 2017). Mass-based quantities show a similar pattern (Table S5) with more variation in $N_{mass}$ explained by LMA than by $V_{cmax}$. However, LMA is negatively related to $N_{mass}$, which indicates that the concentration of N in bulk leaf tissue is lower than that in the photosynthetic machinery.

The coordination hypothesis implies that $V_{cmax}$, and therefore also the metabolic component of $N_{area}$, should be determined by photosynthetic demand – rather than $V_{cmax}$ being determined by $N_{area}$, as is currently assumed in many models. This point is further addressed by (Maire et al. 2012; Dong et al. 2017), who noted that the co-ordination hypothesis predicts an optimal value for metabolic $N_{area}$ on the basis of environmental conditions alone. The empirical $V_{cmax}$–$N_{area}$ relationship has been widely interpreted as a manifestation of ‘N limitation’ of photosynthesis at the leaf level (Luo et al. 2004), and underpins the use of $N_{area}$ as a predictor of photosynthetic capacity in models (Ciais et al. 2014). An alternative interpretation is that leaf metabolic N depends primarily on the demand for photosynthetic capacity, which is set by the local environment and in turn determines the capacity for dark respiration; while N availability primarily influences the allocation of carbon to leaves versus other organs (LeBauer & Treseder 2008; Poorter et al. 2012).

**Conclusions**

The observed thermal acclimation of $R_d$ follows the optimization of $V_{cmax}$ as predicted by the coordination hypothesis. This acclimation dampens the enzyme-kinetic response of $R_d$ to temperature and shows little influence from other factors. The discrepancy between thermal acclimation and enzyme-kinetic thermal response implies that both $R_d$ or $V_{cmax}$, converted to 25°C or any other arbitrarily chosen
reference temperature, must decline with plant growth temperature. These principles would be straightforward to incorporate in an LSM framework, as an alternative to PFT-based schemes in current use. The theory provides an explanation for the observed correlations between $N_{\text{area}}$, $V_{\text{cmax}}$, 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 the terrestrial carbon and nitrogen cycles.

References


Table 1: Summary of Ordinary Least-Squares regressions for natural log-transformed area-based leaf dark respiration ($R_d$), area-based maximum carboxylation rate ($V_{\text{cmax}}$) and their ratio as a function of growth temperature. Both $R_d$ and $V_{\text{cmax}}$ have been standardized to growth temperature ($R_{d,tg}$ and $V_{\text{cmax,tg}}$) and to 25 °C ($R_{d,25}$ and $V_{\text{cmax,25}}$), and normalized by site-mean leaf absorbed photosynthetic photon flux density. The fitted coefficient and its confidence interval are shown together with the corresponding theoretical expectation, the intercept (mean ± standard error), the coefficient of determination ($r^2$) and the degrees of freedom ($df$). 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 ± standard error)</th>
<th>$r^2$</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{d,\text{tg}}$</td>
<td>0.037</td>
<td>0.033</td>
<td>0.029</td>
<td>-9.335±0.051</td>
<td>0.34</td>
<td>1245</td>
</tr>
<tr>
<td>$V_{\text{cmax,\text{tg}}}$</td>
<td>0.055</td>
<td>0.056</td>
<td>0.050</td>
<td>-6.386±0.054</td>
<td>0.50</td>
<td>1009</td>
</tr>
<tr>
<td>$R_{d,\text{tg}}/V_{\text{cmax,\text{tg}}}$</td>
<td>-0.018</td>
<td>-0.020</td>
<td>-0.026</td>
<td>-2.916±0.060</td>
<td>0.05</td>
<td>1007</td>
</tr>
<tr>
<td>$R_{d,25}$</td>
<td>-0.044</td>
<td>-0.049</td>
<td>-0.045</td>
<td>-7.261±0.052</td>
<td>0.25</td>
<td>1245</td>
</tr>
<tr>
<td>$V_{\text{cmax,25}}$</td>
<td>-0.044</td>
<td>-0.044</td>
<td>-0.049</td>
<td>-3.907±0.054</td>
<td>0.26</td>
<td>1009</td>
</tr>
<tr>
<td>$R_{d,25}/V_{\text{cmax,25}}$</td>
<td>0</td>
<td>-0.005</td>
<td>-0.010</td>
<td>-3.317±0.060</td>
<td>0.00</td>
<td>1007</td>
</tr>
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</table>
Table 2: Summary statistics for Ordinary Least Squares regressions of area-based leaf dark respiration ($R_d$) against area-based maximum carboxylation rate ($V_{\text{cmax}}$) assessed at growth temperature and at 25°C. The fitted slopes and intercept are shown together with their standard errors (SE), the coefficient of determination ($r^2$) and the degrees of freedom ($df$).

<table>
<thead>
<tr>
<th>Formula</th>
<th>Slope</th>
<th>SE</th>
<th>Intercept</th>
<th>SE</th>
<th>$r^2$</th>
<th>$p$ value</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_d,\text{tg} \sim V_{\text{cmax},\text{tg}}$</td>
<td>0.015</td>
<td>0.001</td>
<td>0.495</td>
<td>0.026</td>
<td>0.25</td>
<td>&lt;0.05</td>
<td>1007</td>
</tr>
<tr>
<td>$R_d,25 \sim V_{\text{cmax},25}$</td>
<td>0.014</td>
<td>0.001</td>
<td>0.787</td>
<td>0.048</td>
<td>0.16</td>
<td>&lt;0.05</td>
<td>1007</td>
</tr>
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Table 3: Summary statistics for Ordinary Least Squares regressions of area-based leaf nitrogen content against area-based leaf dark respiration ($R_d$), area-based leaf maximum capacity of carboxylation ($V_{cmax}$) assessed at growth temperature and 25°C, and leaf mass per area (LMA). The fitted slopes (mean ± standard error) 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>Coefficient</th>
<th>Intercept</th>
<th>$r^2$</th>
<th>$df$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{cmax, t_g}$</td>
<td>0.083±0.019</td>
<td>0.040±0.059</td>
<td>0.02</td>
</tr>
<tr>
<td>$V_{cmax, 25}$</td>
<td>0.058±0.015</td>
<td>0.491±0.021</td>
<td>−1.849±0.107</td>
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<tr>
<td>$R_d, t_g$</td>
<td>0.242±0.022</td>
<td>−0.199±0.081</td>
<td>0.12</td>
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<tr>
<td>$R_d, 25$</td>
<td>0.148±0.018</td>
<td>0.458±0.021</td>
<td>−2.055±0.106</td>
</tr>
<tr>
<td>LMA</td>
<td>0.177±0.022</td>
<td>0.107±0.018</td>
<td>0.677±0.015</td>
</tr>
<tr>
<td></td>
<td>0.301±0.022</td>
<td>0.508±0.020</td>
<td>−1.724±0.097</td>
</tr>
<tr>
<td></td>
<td>0.174±0.019</td>
<td>0.471±0.020</td>
<td>−1.606±0.097</td>
</tr>
</tbody>
</table>
Figure 1: Schematic of the thermal sensitivities of area-based maximum capacity of 
carboxylation ($V_{cmax}$) and leaf dark respiration ($R_d$). $\beta_{av}$: the acclimated thermal 
sensitivity of $V_{cmax}$ assessed at growth temperature ($T_g$). $\beta_{aR}$: the acclimated thermal 
sensitivity of $R_d$ assessed at growth temperature. $\beta_{IV}$: the kinetic thermal sensitivity of 
$V_{cmax}$ assessed at growth temperature. $\beta_{IR}$: the kinetic thermal sensitivity of $R_d$ assessed 
at growth temperature. $\beta_{av25}$: the acclimated thermal sensitivity of $V_{cmax}$ assessed at 
25°C. $\beta_{aR25}$: the acclimated thermal sensitivity of $R_d$ assessed at 25°C. $\beta_b$: the thermal 
sensitivity of the ratio of $R_d$ to $V_{cmax}$ assessed at growth temperature. $V_{cmax}$ and $R_d$ are 
assessed at growth temperature ($V_{cmax,tg}$ and $R_{d,tg}$) as a starting point, 25°C as the 
common practice ($V_{cmax,25}$ and $R_{d,25}$) and a higher growth temperature ($T_g'$) for the 
acclimation behaviour ($V_{cmax,tg}'$, $R_{d,tg}'$, $V_{cmax,25}'$ and $R_{d,25}'$) to warming.

\[
\begin{align*}
\ln V_{cmax} & = 5.5\%K^{-1} (Eqn.2) \\
\ln R_d & = 8.1\%K^{-1} (Heskel2016) \\
\beta_{av25} = & \beta_{av} - \beta_{aR25} = -4.4\%K^{-1} (Eqn. 2) \\
\beta_{aR25} = & \beta_{aR} - \beta_{IR25} = 3.7\%K^{-1} (K&K2007) \\
\beta_{ir} = & \beta_{IR} - \beta_{iV} = -1.8\%K^{-1} \\
\beta_{aV25} = & \beta_{av} + \beta_b = 3.7\%K^{-1} \\
\beta_{iR25} = & \beta_{iR} = -4.4\%K^{-1}
\end{align*}
\]
Figure 2: Natural log-transformed light-normalized leaf dark respiration ($R_d$), maximum carboxylation rates ($V_{\text{max}}$) and their ratio, standardized to growth temperature ($R_{\text{d,lg}}, V_{\text{max,lg}}$, $b$: $R_{\text{d,lg}}/V_{\text{max,lg}}$) and to 25°C ($R_{\text{d,25}}, V_{\text{max,25}}, b_2$: $R_{\text{d,25}}/V_{\text{max,25}}$), as a function of growth temperature (mGDD). Solid blue lines are the fitted lines from Ordinary Least Squares regressions. Solid black lines are theoretical predictions. Dashed lines represent the instantaneous temperature response based on enzyme kinetics.