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**Title:** Macromolecular Rate Theory (MMRT) Provides a Thermodynamics Rationale to Underpin the Convergent Temperature Response in Plant Leaf Respiration

**Running Title:** MMRT explains thermal response of respiration

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**Abstract:** Temperature is a crucial factor in determining the rates of ecosystem processes, e.g. leaf respiration ( $R$ ) – the flux of plant respired  $\text{CO}_2$  from leaves to the atmosphere. Generally,  $R$  increases exponentially with temperature and formulations such as the Arrhenius equation are widely used in earth system models. However, experimental observations have shown a consequential and consistent departure from an exponential increase in  $R$ . What are the principles that underlie these observed patterns? Here, we demonstrate that macromolecular rate theory (MMRT), based on transition state theory for enzyme-catalyzed kinetics, provides a thermodynamic explanation for the observed departure and the convergent temperature response of  $R$  using a global database. Three meaningful parameters emerge from MMRT analysis: the temperature at which the rate of respiration would theoretically reach a maximum (*the optimum temperature,  $T_{opt}$* ), the temperature at which the respiration rate is most sensitive to changes in temperature (*the inflection temperature,  $T_{inf}$* ) and the overall curvature of the log(rate) versus temperature plot (*the change in heat capacity for the system,  $\Delta C_p^\ddagger$* ). On average the highest potential enzyme-catalyzed rates of respiratory enzymes for  $R$  is predicted to occur at  $67.0 \pm 1.2$  °C and the maximum temperature sensitivity at  $41.4 \pm 0.7$  °C from MMRT. The average curvature (average negative  $\Delta C_p^\ddagger$ ) was  $-1.2 \pm 0.1$  kJ.mol<sup>-1</sup>K<sup>-1</sup>. Interestingly,  $T_{opt}$ ,  $T_{inf}$  and  $\Delta C_p^\ddagger$  appear insignificantly different across biomes and plant functional types (PFTs), suggesting that thermal response of respiratory enzymes in leaves could be conserved. The derived parameters from MMRT can serve as thermal traits for plant leaves that represents the collective temperature response of metabolic respiratory enzymes and could be useful to understand regulations of  $R$  under a warmer climate. MMRT extends the classic transition state theory to enzyme-catalyzed

reactions and provides an accurate and mechanistic model for the short-term temperature response of  $R$  around the globe.

## Introduction

Leaf respiration ( $R$ ) contributes  $c.30$  Pg (30 billion metric tons) carbon per year to the atmosphere (Prentice *et al.*, 2001; Canadell *et al.*, 2007; IPCC, 2013), about 4 times higher than the industrial CO<sub>2</sub> emissions ( $\sim 8$  Pg C yr<sup>-1</sup>) between 2002 to 2011 (IPCC the fifth assessment report Table 6.1, chapter 6 (Ciais *et al.*, 2013)). Consequently, small changes in  $R$  have the potential to make a huge impact on the atmospheric CO<sub>2</sub> concentrations. It is generally predicted that  $R$  will increase with the increasing mean global temperature from the current earth system model projections. Increases in  $R$  are not linear with temperature but rather exponential as modelled by the Arrhenius equation. However, departures from Arrhenius behaviour are well known for many biologically driven reactions such as leaf respiration, photosynthesis and soil carbon decomposition (Lloyd & Taylor, 1994; Tjoelker *et al.*, 2001; Alster *et al.*, 2016; Ma *et al.*, 2017; Robinson *et al.*, 2017).

In a recent study, Heskell *et al.* (2016b) reported a universal convergence in temperature response of  $R$  across different biomes and plant functional types (PFTs) using a global dataset of plant leaf respiration measurements. They demonstrated a consistent curvature in log-transformed  $R$  vs temperature plots that was best fit using a second-order log-polynomial model (LP model) for a large number of  $R$  versus temperature datasets. They compared the LP model to four other conventional models (exponential fixed-Q<sub>10</sub>, Arrhenius, Lloyd & Taylor and variable-Q<sub>10</sub>) and showed improved predictive power in estimating the carbon release from vegetation. An modified Arrhenius model with 3 components, which describes the temperature dependence of activation energy in Arrhenius model as a second-order polynomial function, also provides equivalent fits as the LP model (Kruse & Adams, 2008; Kruse *et al.*, 2011; Adams *et al.*, 2016; Heskell *et al.*, 2016a). Since respiratory metabolism in a leaf involves a series of enzyme-catalyzed reactions, via the tricarboxylic acid (TCA) cycle or cytochrome pathways (Buchanan *et al.*, 2015), a mechanistic underpinning of the temperature response of  $R$  may be found in the temperature dependence of enzyme-catalyzed reaction rates.

We have recently developed macromolecular rate theory (MMRT), which extends the classic transition state theory for the temperature dependence of chemical reactions to those reactions catalyzed by enzymes (large macromolecules, hence the MMRT name) (Hobbs *et al.*, 2013; Arcus *et al.*, 2016). We have also applied MMRT to complex biological systems such as soil processes to describe their temperature dependence, e.g., soil carbon decomposition, nitrification, denitrification, methanogenesis and soil respiration (Schipper *et al.*, 2014; Robinson *et al.*, 2017). MMRT has recently been applied by other groups to soil enzymes and microbial processes in soil (Alster *et al.*, 2016) and has been used to distinguish between nitrifying archaea and bacteria (Taylor *et al.*, 2016). In all of these studies, MMRT captures the curvature in the log(rate) versus temperature plots without invoking enzymatic denaturation. Here, we show that MMRT also models the short-term temperature dependence of  $R$  with predictive power equivalent to the LP model defined by Heskell *et al.* (2016b). Indeed, we show that the two models are nearly equivalent mathematically. Specifically, we use the large  $R$  dataset collected by Heskell and colleagues to compare the performance of Arrhenius, MMRT and LP models in describing the temperature response of  $R$ . We argue that whilst the LP model provides excellent empirical predictions with respect to  $R$ , the MMRT model goes a step further in determining parameters that have a basis in thermodynamics and thus, meaningful interpretation when comparing the temperature dependence of different ecosystem processes at differing scales.

## Materials and methods

### *R* measurements

We used the dataset provided by Heskell *et al.* (2016b), which included 673 individual temperature response curves of  $R$  across 231 species, 18 sites, 7 biomes and 7 plant functional types (PFTs).

Details of the field sites, species, biomes, PFTs and protocols for measurement of CO<sub>2</sub> exchange between leaf and atmosphere are provided in Heskell *et al.* (2016b). In the current study, for consistency, we used respiration data measured up to 45 °C.

## Development of MMRT

Chemical, biological and ecological modellers have long used the Arrhenius function to describe the relationship between temperature and the reaction rate:  $k=A \exp(-E_a/RT)$ , where the  $k$  is the rate constant,  $A$  is the pre-exponential factor,  $E_a$  is the activation energy,  $R$  is the gas constant and  $T$  is temperature (K). The central concept invoked by the Arrhenius function is the activation energy ( $E_a$ ) for a reaction which is defined as the energy barrier between the ground state and transition state for a given reaction. Eyring, Evans, Polanyi and others extended the Arrhenius function to develop Transition State Theory (TST) that provides a statistical thermodynamic description of the pre-exponential factor ( $A$ ) and defines  $E_a$  as the difference in Gibbs free energy between the ground state and transition state ( $\Delta G^\ddagger$ ). Further, it is generally assumed that the temperature dependence of  $\Delta G^\ddagger$  is described by the Gibbs equation,  $\Delta G^\ddagger=\Delta H^\ddagger - T\Delta S^\ddagger$ , where  $\Delta H^\ddagger$  is the change in enthalpy, and  $\Delta S^\ddagger$  is the change in entropy, between the ground state and the transition state for the reaction. Hence, the Eyring equations and their equivalent log forms are:

$$k = \frac{\kappa k_B T}{h} e^{\left(\frac{-\Delta G^\ddagger}{RT}\right)}, \quad \ln(k) = \ln\left(\frac{\kappa k_B T}{h}\right) - \frac{\Delta G^\ddagger}{RT} \quad (1)$$

$$k = \frac{\kappa k_B T}{h} e^{\left[\frac{-(\Delta H^\ddagger - T\Delta S^\ddagger)}{RT}\right]}, \quad \ln(k) = \ln\left(\frac{\kappa k_B T}{h}\right) - \left[\frac{(\Delta H^\ddagger - T\Delta S^\ddagger)}{RT}\right] \quad (2)$$

where  $\kappa$ ,  $k_B$ ,  $h$  and  $R$  refer to the transmission coefficient (here,  $\kappa$  is assumed to be 1 for simplicity), Boltzmann and Planck's constants, and the universal gas constant, respectively. Similarly, it is generally assumed that  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$  are independent of temperature and this assumption holds for the vast majority of chemical reactions involving small molecules in standard solvents. However, in biological systems when enzymes (macromolecules) are involved in the reactions, this assumption no longer holds and we must consider the change in heat capacity ( $\Delta C_P^\ddagger$ ) for the reaction (formally, the temperature dependence of the enthalpy and entropy for the reaction). The  $\Delta C_P^\ddagger$  has been shown to be important in enzyme catalysis (Arcus & Pudney, 2015; Arcus *et al.*, 2016). This leads to an expansion of equation (2) above to give the MMRT equation:

$$\ln(k) = \ln\left(\frac{\kappa k_B T}{h}\right) - \frac{(\Delta H_{T_0}^\ddagger + \Delta C_P^\ddagger(T - T_0))}{RT} + \frac{(\Delta S_{T_0}^\ddagger + \Delta C_P^\ddagger(\ln T - \ln T_0))}{R} \quad (3)$$

where  $T_0$  is a suitable reference temperature and  $\Delta C_p^\ddagger$  is the change in heat capacity between the ground state and the transition state for the enzyme-catalyzed reaction. Although the MMRT function appears complicated, it is nothing more than a theoretical parameterization of the pre-exponential term ( $A$ ) and the activation energy ( $E_a$ ) from the familiar Arrhenius function. If there is no heat capacity change during the reaction (i.e.,  $\Delta C_p^\ddagger = 0$ ), MMRT simply collapses to the Arrhenius and Eyring equations (Hobbs *et al.*, 2013; Arcus *et al.*, 2016). Notably, the Arrhenius function has two unknowns ( $A$  and  $E_a$ ) and the MMRT function has three unknowns ( $\Delta H_{T_0}^\ddagger$ ,  $\Delta S_{T_0}^\ddagger$  and  $\Delta C_p^\ddagger$ ). However,  $\Delta H_{T_0}^\ddagger$  and  $\Delta S_{T_0}^\ddagger$  are tightly correlated due to the so called enthalpy-entropy compensation (Fig. S1) and hence, adding a third parameter does not simply improve the fit to the data *ad hoc*. Indeed, the log-polynomial function used by Heskell and colleagues has three *independent* unknowns (the coefficients,  $a$ ,  $b$ , and  $c$ ). Additionally, the  $\Delta S_{T_0}^\ddagger$  in MMRT captures the magnitude change of reaction rate caused by substrate concentrations.

The temperature dependence of enzyme-catalyzed rates typically shows an exponential rise with temperature up to an optimum temperature above which the rate declines. The textbook explanation for the decline in rate at high temperatures is denaturation of the enzyme leading to its inactivation. However, it has been demonstrated in very many cases that this does not account for either the optimum temperature ( $T_{opt}$ ) or curvature in the log(rate) versus temperature plots for temperatures below  $T_{opt}$  (Thomas & Scopes, 1998; Buchanan *et al.*, 1999; Daniel & Danson, 2010; Hobbs *et al.*, 2013). We have shown that enzyme-catalyzed rates proceed with a measurable  $\Delta C_p^\ddagger$  and that the curvature below  $T_{opt}$  and the position of both  $T_{opt}$  and an inflexion point  $T_{inf}$  can be deduced from MMRT and the important parameter  $\Delta C_p^\ddagger$  (Hobbs *et al.*, 2013; Arcus *et al.*, 2016) (see Text S1 for details about the derived parameters  $T_{opt}$  and  $T_{inf}$  from MMRT).

### The mathematical connection between MMRT and the LP model

Heskel *et al.* (2016b) plot the log of the rate versus temperature for leaf respiration and fit a second order polynomial to the data showing excellent convergence of the polynomial coefficients across ecosystems and plant functional types (the coefficients  $b$  &  $c$  converge, the third coefficient,  $a$ , determines the absolute amplitude of the rate at a reference temperature which varies between species and climates).

$$\ln(k) = a + bT + cT^2 \quad (4)$$

We have also fitted the MMRT function to these data and find a similar convergence of the MMRT parameters (see next section for details). This suggested to us that the LP function and MMRT are mathematically closely related (Fig. 1) and this turns out to be the case.

A Taylor expansion for Eq (1) around a suitable reference temperature  $T_0$  gives (see Text S2 for the deduction):

$$\ln(k) = \ln\left(\frac{k_B T_0}{h}\right) - \frac{\Delta G_{T_0}^\ddagger}{RT_0} + \left(\frac{1}{T_0} + \frac{\Delta H_{T_0}^\ddagger}{RT_0^2}\right)(T - T_0) + \left(\frac{\Delta C_P^\ddagger}{2RT_0^2}\right)(T - T_0)^2 \quad (5)$$

The first two terms are a constant and may be combined as coefficient  $a$  in Eq (4) above. Importantly, these terms define the rate at the reference temperature and encapsulate all of the variables that contribute to that rate (e.g., substrate availability, activation energy at  $T_0$ , moisture availability, etc.).

This is the amplitude term and in keeping with Heskel and colleagues, we will call this  $a(\text{MMRT})$ .

The second two terms constitute the linear coefficient of  $T$  (equivalent to coefficient  $b$  in Eq (4) above). The last term is the quadratic term (coefficient  $c$  in Eq (4) above). Thus, Eq (4) used by

Heskel *et al.* (2016b) can be rewritten as:

$$\ln(k) = a + b(T - T_0) + c(T - T_0)^2 \quad (6)$$

Hence the correspondence between MMRT and the LP function is:

$$a = \ln\left(\frac{k_B T_0}{h}\right) - \frac{\Delta G_{T_0}^\ddagger}{RT_0}, \quad b = \frac{1}{T_0} + \frac{\Delta H_{T_0}^\ddagger}{RT_0^2}, \quad c = \frac{\Delta C_P^\ddagger}{2RT_0^2} \quad (7)$$

Thus, the amplitude term,  $a$  is simply Eqs 1&5 at the reference temperature  $T_0$ . The linear term ( $b$ ) is a function of the change in enthalpy for the reaction at the reference temperature ( $\Delta H_{T_0}^\ddagger$ ) and the quadratic term ( $c$ ) is a function of the change in heat capacity for the reaction ( $\Delta C_p^\ddagger$ ) and defines the “curvature” of the rate versus temperature.

#### *Curve fitting and statistics*

In this study, we fitted the leaf respiration rate versus temperature datasets using Arrhenius, MMRT and LP functions to retrieve the estimated parameters from each model. Before fitting the data, we checked each individual  $\ln R$ - $T$  curve manually by plotting in Matlab (2015a (The MathWorks Inc., Natick, MA, USA). In several cases we identified an unexplained upward rise in respiration rate at low temperatures (below  $\sim 10^\circ\text{C}$ ). This low temperature hook may be due to the measurement protocols in leaf respiration where the temperature adjustment period was insufficiently long to fully cool the leaves before temperature began to increase in the cuvette. About 7% of the  $\ln R$ - $T$  dataset curves showed this phenomenon and to minimize the effects of this artefact on the fitted parameters, we fitted measurements to data above this low temperature using Arrhenius, LP and MMRT functions.

For each curve-fitting run, the parameters (Arrhenius pre-exponential factor,  $A$ , and activation energy  $E_a$ ; MMRT,  $\Delta H_{T_0}^\ddagger$ ,  $\Delta S_{T_0}^\ddagger$  and  $\Delta C_p^\ddagger$ ; LP coefficients,  $a$ ,  $b$  and  $c$ ) were not constrained. The  $T_0$  was set to 298.15 K ( $25^\circ\text{C}$ ) as a reference temperature. The curve was fitted using the *nlinfit* function of MATLAB 2015a (The MathWorks Inc., Natick, MA, USA). We conducted 1000-iteration bootstrapping with sample replacement to retrieve the estimated parameters from three models. At each bootstrapping run, we constrained 75% of the data in each  $\ln R$ - $T$  curve since more data results in higher confidence in parameter estimates (Robinson *et al.*, 2017). We use the medians of the 1000-iteration bootstrapped parameters to represent the best estimates. For model comparisons among three models, we further calculated the corrected Akaike Information Criterion (AICc) value to assess the performance of the three models. AICc provides a measure for model comparison and suggests that a model showing a smaller AICc value is better. We used 3 parameters in MMRT and LP models and 2 parameters for the Arrhenius function to calculate the AICc. We applied one-way ANOVA to test the difference of the calculated AICc among three models across 673 curves.



Mean parameter values of each species for MMRT, i.e.,  $\Delta H_{T_0}^\ddagger$ ,  $\Delta S_{T_0}^\ddagger$  and  $\Delta C_P^\ddagger$ , were calculated, including 231 species in 673 individual measurements across the global dataset. We further calculated  $T_{\text{opt}}$  and  $T_{\text{inf}}$  only when they were within the biological range (298.15-373.15K, 25-100 °C). The mean  $a(\text{MMRT})$ ,  $\Delta H_{T_0}^\ddagger$ ,  $\Delta C_P^\ddagger$ ,  $T_{\text{opt}}$  and  $T_{\text{inf}}$  were statistically compared across 7 biomes and 7 plant functional types (PFTs) using mixed effect model as Heskell *et al.* (2016b). The Tukey's honestly significant difference (HSD) test was used to conduct the *post hoc* intra-group comparisons.

## Results

### *Comparisons between fits using Arrhenius, MMRT and LP equations*

All three models, Arrhenius (Eq.1), MMRT (Eq.3) and LP (Eq.6) were able to describe the temperature response of  $R$  (Fig. 2a), although it is clear from the residuals that the MMRT and LP models outperform the Arrhenius function (Fig. 2b). ANOVA of AICc among 3 models also showed that both MMRT and LP models were consistently better than the Arrhenius model in predicting the temperature response of  $R$  across 673 individual  $\ln R$ - $T$  curves, with statistically significant lower AICc ( $p < 0.0001$ ) values from both MMRT and LP models compared to the Arrhenius function (Fig. 2c). This conclusion is consistent with the results of Heskell *et al.* (2016b), who demonstrated that the LP model better characterized the temperature response of  $R$  when compared to four other Arrhenius-based models. MMRT provided equivalent predictive power for the temperature response of  $R$  when compared to the empirical LP model since there was no statistical difference among AICc values ( $p = 0.99$ ) between MMRT and LP models. This is unsurprising given the near equivalence of the mathematical functions for the MMRT and LP models or a modified Arrhenius model proposed by Adams *et al.* (2016).

### *Bridging MMRT and polynomial models*

The parameters derived from fits between the LP and MMRT models are nearly identical (Fig. 3). The  $R$  value at  $T_0$  (298.15K, 25°C), i.e., parameter  $a$  in the LP model, is almost the same as that calculated from MMRT at  $T_0$  (Fig. 3a), with an inconsequential difference between  $a$  and  $R$  at  $T_0$  ( $a - R_{25}$ ) of  $-0.0011 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Parameter  $b$  in the LP model, is commensurate with that from MMRT (Fig. 3b), with a difference of  $9.1 \times 10^{-5} \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ K}^{-1}$ . The curvature term between

MMRT and LP is also equivalent (Fig. 3c). The difference between  $c$  and  $\Delta C_p^\ddagger / 2RT_0^2$  is  $-2.2 \times 10^{-4}$   $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ K}^{-2}$ , which is equivalent to a difference of  $-0.3 \text{ kJ mol}^{-1} \text{ K}^{-1}$  in  $\Delta C_p^\ddagger$ . Thus, MMRT and LP functions both model  $R$  equally (Fig. 1) and provide closely comparable parameters, suggesting that we can either use MMRT or the LP function to characterize the temperature response of  $R$ .

*MMRT explains the temperature response of R*

MMRT provides three biologically meaningful parameters: the temperature at which the rate of respiration is predicted to reach maximum rates (the so called *optimum temperature*,  $T_{\text{opt}}$ ), the temperature at which the respiration rate is most sensitive to changes in temperature (the *inflection temperature*,  $T_{\text{inf}}$ ) and the overall curvature of the  $\ln R$ -T curve (the so called *change in heat capacity* for the system,  $\Delta C_p^\ddagger$ ). The  $T_{\text{opt}}$  and  $T_{\text{inf}}$  are a function of  $\Delta C_p^\ddagger$  and  $\Delta H_{T_0}^\ddagger$  (See Text S1).  $\Delta G_{T_0}^\ddagger$  ( $\Delta G_{T_0}^\ddagger = \Delta H_{T_0}^\ddagger - T_0 \Delta S_{T_0}^\ddagger$ ) reflects the magnitude of  $R$  at the reference temperature. It must be noted that many variables are rolled into  $\Delta G_{T_0}^\ddagger$  and it cannot be considered a true activation energy. To make this point clear, we will refer to the magnitude term, the first two terms from Eq.7, as  $a(\text{MMRT})$ . The convergent MMRT parameters for temperature response of  $R$  are  $\Delta C_p^\ddagger$  and  $\Delta H_{T_0}^\ddagger$ , which are the analogues of the parameters  $c$  and  $b$  from Heskell *et al.* (2016b), respectively. Accordingly, there is no significant difference in  $\Delta H_{T_0}^\ddagger$  across biomes ( $p=0.72$ ) and plant functional types (PFTs) ( $p=0.60$ ) or in  $\Delta C_p^\ddagger$  ( $p=0.22$  and  $p=0.24$  in biomes and PFTs, respectively). The global mean parameters,  $b$  and  $c$  in Heskell *et al.* (2016b),  $0.1012 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ C}^{-1}$  and  $-0.0005 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ C}^{-2}$ , are equivalent to  $\Delta H_{T_0}^\ddagger = 53.8 \text{ kJ mol}^{-1}$  and  $\Delta C_p^\ddagger = -0.7 \text{ kJ mol}^{-1} \text{ K}^{-1}$  in MMRT, respectively. The results from MMRT agree with the convergent temperature response of  $R$  in Heskell *et al.* (2016) using the LP model. The consequence of consistent  $\Delta H_{T_0}^\ddagger$  and  $\Delta C_p^\ddagger$  values leads to insignificant differences of  $T_{\text{opt}}$  and  $T_{\text{inf}}$  of  $R$  across global datasets (Table 1). We found marginal differences in  $T_{\text{opt}}$  and  $T_{\text{inf}}$  across biomes ( $p=0.07$  and  $p=0.09$ ) and no statistically difference across PFTs ( $p=0.32$  and  $p=0.42$ ), with mean  $T_{\text{opt}}$  and  $T_{\text{inf}}$  of  $R$   $67.0 \text{ }^\circ\text{C}$  and  $41.4 \text{ }^\circ\text{C}$ , respectively. While we were unable to demonstrate statistically

significant differences in  $T_{\text{opt}}$ ,  $T_{\text{inf}}$  within biomes and PFTs, ranges in both were high (Table 1) and further work is needed to determine the reasons for these large ranges.

In contrast, the magnitude term,  $a(\text{MMRT})$ , was significantly different between biomes ( $p < 0.0001$ ) and PFTs ( $p < 0.0001$ ) (Table 1), with a decreasing trend from tundra (Tu) to tropical rainforest at low elevation (TrRF\_1w) and from  $C_3$  herbaceous ( $C_3H$ ) plant to broadleaf evergreen tropical (BIEvTrp) plants (Table 1). Regression analysis revealed that both mean annual temperature (MAT) ( $R^2 = 0.24$ ,  $p = 0.037$ ) and mean annual precipitation (MAP) ( $R^2 = 0.74$ ,  $p < 0.0001$ ) was correlated with  $a(\text{MMRT})$  across 18 sites at global scales (Fig. S2). Our results also identified a systematic variation in  $a(\text{MMRT})$  with water availability (Fig 4.a), showing a clear negative relationship with aridity index ( $R^2 = 0.65$ ,  $p < 0.0001$ ). Our result agrees with the acclimation pattern of plant respiration that shows a similar  $R$  rate for plants from contrasting environments (Fig. 4b) (Atkin & Tjoelker, 2003; Atkin *et al.*, 2015; Vanderwel *et al.*, 2015).

## Discussion

We have compared the predictive power of MMRT, LP and Arrhenius models for characterizing the temperature response of plant leaf respiration ( $R$ ) using the short-term temperature-response data reported by Heskell and colleagues (Heskell *et al.*, 2016b) across different biomes and plant functional types (PFTs). Our results show that both MMRT and LP functions are better than the Arrhenius model in characterizing the temperature response of  $R$ . These results are consistent with our expectation across different biomes and PFTs from the global dataset, suggesting a convergence in temperature response of  $R$  as shown by Heskell *et al.* (2016b) using the LP model. MMRT and LP models have equivalent explanatory power for predicting the temperature response of  $R$ , and we have shown here the mathematical equivalence between these two models. We now explore the differences and utility of the MMRT and LP models.

The LP model is straightforward to understand and implement as it has a simple and familiar mathematical form. The initial increase and then decline of  $R$  with increasing temperature is determined by the curvature term, i.e.,  $c$  in Eq (6), and the other 2 parameters, i.e.,  $a$  and  $b$ , help to constrain the magnitude and changing rate of  $R$  with temperature. From a modelling perspective, this LP model is sufficient to describe the temperature response of  $R$ . However, the lack of biological

meaning of the fitted parameters of the LP model limits its capability to reveal the regulation of temperature response of plant  $R$ . As an alternative, MMRT incorporates the central concepts of thermodynamics (activation energies, enthalpy and heat capacity) which will allow insight into the determinants of respiration pathways for  $R$  and potential comparison to temperature dependence of other biological processes, such as photosynthesis and respiration by other groups of organisms, e.g., soil respiration (Robinson *et al.*, 2017), by characterizing their thermal properties using the changed heat capacity,  $\Delta C_p^\ddagger$ . This may allow a unified understanding of the temperature response of biological processes. For example, the average curvature ( $\Delta C_p^\ddagger = -1.2 \pm 0.1 \text{ kJ} \cdot \text{mol}^{-1} \text{K}^{-1}$ ) for plant respiration is significantly less than the average curvature seen for heterotrophic soil respiration accurately measured in the laboratory ( $\Delta C_p^\ddagger = -2.1 \pm 0.1 \text{ kJ} \cdot \text{mol}^{-1} \text{K}^{-1}$ ) (Robinson *et al.*, 2017).

MMRT may appear to have a more complicated form but it is simply the form used to model the temperature dependence of enzyme-catalyzed rates. It has its roots in transition state theory (TST) which is a very robust description of chemical reaction rates. Biologists and ecologists have observed decreasing activities of the leaf respiratory enzymes at higher temperatures and modified the Arrhenius equation to explore the mechanism by introducing a polynomial term to describe the temperature dependence of activation energy (Kruse & Adams, 2008; O'Sullivan *et al.*, 2013; Noguchi *et al.*, 2015; Heskell *et al.*, 2016b). We have shown that MMRT can be rearranged to a form equivalent to the LP function using a 2nd-order Taylor expansion (Eq.5). MMRT also collapses to the Arrhenius (and TST) function when the  $\Delta C_p^\ddagger = 0$ . It is well known that the activation energy that describes ecosystem processes is temperature dependent (Lloyd and Taylor, 1994; Davidson & Janssens, 2006). MMRT accounts for this temperature dependence by introducing the concept of  $\Delta C_p^\ddagger$ , the change in heat capacity between the ground state and transition state of enzyme-substrate complex (Hobbs *et al.*, 2013; Arcus & Pudney, 2015). Formally,  $\Delta C_p^\ddagger$  is defined as the temperature dependence of the enthalpy and entropy and thus encapsulates the temperature dependence of the activation energy. The molecular origins of  $\Delta C_p^\ddagger$  have been discussed elsewhere (Arcus & Pudney, 2015; Arcus *et al.*, 2016). Briefly, the chemical meaning of  $\Delta C_p^\ddagger$  is to indicate the difficulty or the energy barrier needed to be crossed for enzyme-catalyzed reactions to proceed. As a reaction gets more difficult, a higher absolute  $\Delta C_p^\ddagger$  can be observed.  $\Delta C_p^\ddagger$  values are generally negative for

enzyme-catalyzed reactions and it also can be scaled up to describe enzyme-driven processes such as metabolism. Here, we demonstrate that the majority of  $R$  curves have negative  $\Delta C_p^\ddagger$  with an average value of  $-1.2 \pm 0.1 \text{ kJ.mol}^{-1}\text{K}^{-1}$  for 167 out of 231 species (70%). The consequence of a negative  $\Delta C_p^\ddagger$  is that the catalyzed rate will diverge from Arrhenius behaviour and predict an optimum temperature above which rates will decline (although this predicted optimum is above observed temperatures in nature). Similarly improved fits by MMRT to temperature response have also been demonstrated for enzymes kinetics (Hobbs *et al.*, 2013), soil microbial extracellular enzymes (Alster *et al.*, 2016), soil nitrification (Taylor *et al.*, 2016) and soil respiration (Schipper *et al.*, 2014; Robinson *et al.*, 2017). Heskell *et al.* (2016b) argued for a universal convergence of temperature response of  $R$  using the same global leaf respiration dataset and the current study shows that MMRT is also able to characterize the temperature response of  $R$ . From the enzyme kinetic perspective, the constant observed  $\Delta C_p^\ddagger$  across the globe for leaf respiration (Table 1) suggests that the contributions from metabolic enzyme rates for leaf respiration across different plant species are similar, supporting the concept of a global convergence of the short-term temperature response of  $R$  (Heskell *et al.*, 2016b).

Based on the short-term measurements of  $R$ , plants across biomes are adapted to their respective environments and homeostasis of respiration could result in a similar  $R$  rate for plants from contrasting environments (Fig. 4b) (Atkin & Tjoelker, 2003; Ow *et al.*, 2008a, 2008b; Slot & Kitajima, 2015). For example, the  $R$  rates from tropical forests have values close to those of tundra plants (i.e. respiratory homeostasis), when each is measured at their respective growth temperature (Fig. 4b). This would then correspond to decreasing  $a(\text{MMRT})$  from cool/dry to high temperature/humid environment (Fig. 4b). The pattern of  $a(\text{MMRT})$  calculated from MMRT agrees with previous findings (Atkin *et al.*, 2015; Vanderwel *et al.*, 2015) which showed a clear negative relationship between  $R$  at reference temperature and aridity index. When comparisons were made of rates of respiration at the prevailing growth temperature of each site, we demonstrated a similar  $R$  among contrasting environments (Fig. 4b). Interestingly, site-to-site variations in mean annual precipitation were more strongly correlated with  $a(\text{MMRT})$  than MAT, suggesting a higher  $a(\text{MMRT})$  in the arid regions than those in more humid climates (Fig. S2). Thus, for a given growth temperature, exposure to dry conditions is associated with higher basal rates of respiration (i.e. higher  $a(\text{MMRT})$ ). Similarly,

for a given MAP,  $R$  decreases with increasing growth temperature. These patterns are similar to those reported by Atkin *et al.* (2015) in their analysis of global variations in leaf respiration at a common measuring temperature of 25°C.

We found that the  $T_{\text{opt}}$  derived from MMRT was generally greater than the measured  $T_{\text{max}}$  (the actual maximum  $R$  based on measurements reported by O'Sullivan *et al.* (2017)) for the same plant species.  $T_{\text{max}}$  exhibited clear biogeographic patterns with  $T_{\text{max}}$  increasing linearly from polar to equatorial regions (O'Sullivan *et al.*, 2017). Similar patterns for  $T_{\text{opt}}$  were not found and our results suggested a relatively constant  $T_{\text{opt}}$  across all the observed species from the globe (Table1). We hypothesize that the difference between  $T_{\text{opt}}$  and  $T_{\text{max}}$  was due to the way these were determined and the underlying physiological responses these two indices represent.  $T_{\text{opt}}$  was mathematically determined from fits of MMRT using respiration measurements up to 45 °C, whereas,  $T_{\text{max}}$  was determined experimentally with leaves exposed to temperatures often well above 50 °C (O'Sullivan *et al.*, 2017). The  $T_{\text{opt}}$  derived from MMRT depends on thermodynamic properties of contributing enzymes, particularly the  $\Delta C_p^\ddagger$  value of the enzymes involved in leaf respiration and this parameter was very tightly constrained across biomes. The  $T_{\text{opt}}$  retrieved from MMRT represents the temperature where enzymes reached their theoretical maximum rate of catalysis in the absence of other biochemical constraints. The measured  $T_{\text{max}}$ , also includes other factors that can contribute to reduction in the overall rate of respiration, e.g., change in cell membrane properties (Schrader *et al.*, 2004), respiration being uncoupled from mitochondrial electron transport (Skulachev, 1998; Hüve *et al.*, 2011), or increased drought stress (Atkin & Macherel, 2009) at high temperature. These factors lead to a 'burst' of  $R$  around 47°C (O'Sullivan *et al.*, 2013), that varies between species and is presumably due to variation of other leaf traits, e.g., leaf size (Wright *et al.*, 2017), than the enzymes involved in respiration. Hence, we hypothesize that the  $T_{\text{opt}}$  from MMRT and the measured  $T_{\text{max}}$  describe the temperature response of  $R$  at level of the contributing metabolic enzymes and at the whole leaf level, respectively. A higher  $T_{\text{opt}}$  than  $T_{\text{max}}$  suggests a higher thermal tolerance of respiratory enzymes than the whole leaf. If this hypothesis is correct it argues that thermal response of respiratory enzymes in leaves are highly conserved while leaves adapt to different climates by varying leaf traits, such as leaf size, which demonstrates a clear consistent latitudinal gradient, e.g.,

large-leaved species predominate in wet, hot, sunny environments (Wright *et al.*, 2017). This conserved temperature response of plant leaf respiration across geophysical gradient or evolutionary scale is worth further exploring. Nevertheless, MMRT provides a tool to explore the thermodynamic properties of respiratory enzymes. The information could be useful to understand regulations of  $R$  under a warmer climate and predict the short-term temperature response of  $R$  accurately.

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### References

- Adams MA, Rennenberg H, Kruse J (2016) Different models provide equivalent predictive power for cross-biome response of leaf respiration to temperature. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, E5993–E5995.
- Alster CJ, Baas P, Wallenstein MD, Johnson NG, von Fischer JC (2016) Temperature Sensitivity as a Microbial Trait Using Parameters from Macromolecular Rate Theory. *Frontiers in Microbiology*, **7**, 1821.
- Arcus VL, Pudney CR (2015) Change in heat capacity accurately predicts vibrational coupling in enzyme catalyzed reactions. *FEBS letters*, **589**, 2200–6.
- Arcus VL, Prentice EJ, Hobbs JK et al. (2016) On the temperature dependence of enzyme-catalyzed rates. *Biochemistry*, **55**, 1681–1688.
- Atkin OK, Macherel D (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals of Botany*, **103**, 581–597.
- Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science*, **8**, 343–351.

- Atkin OK, Bloomfield KJ, Reich PB et al. (2015) Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. *New Phytologist*, **206**, 614–636.
- Buchanan CL, Connaris H, Danson MJ, Reeve CD, Hough DW (1999) An extremely thermostable aldolase from *Sulfolobus solfataricus* with specificity for non-phosphorylated substrates. *The Biochemical journal*, **343**, 563–70.
- Buchanan BB, Gruissem W, Jones RL (2015) *Biochemistry & Molecular Biology of Plants*. John Wiley & Sons, Ltd, West Sussex, UK, 634-635 pp.
- Canadell JG, Le Quéré C, Raupach MR et al. (2007) Contributions to accelerating atmospheric CO<sub>2</sub> growth from economic activity, carbon intensity, and efficiency of natural sinks. *Proceedings of the National Academy of Sciences*, **104**, 18866–18870.
- Ciais P, Sabine C, Bala G et al. (2013) *Carbon and Other Biogeochemical Cycles* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, J., Boschung, Nauels A, Xia Y, Bex V, P.M. Midgley). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.
- Daniel RM, Danson MJ (2010) A new understanding of how temperature affects the catalytic activity of enzymes. *Trends in Biochemical Sciences*, **35**, 584–591.
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.
- Heskel MA, Atkin OK, O’Sullivan OS et al. (2016a) Reply to Adams et al.: Empirical versus process-based approaches to modeling temperature responses of leaf respiration. *Proceedings of the National Academy of Sciences*, **113**, E5996–E5997.
- Heskel MA, O’Sullivan OS, Reich PB et al. (2016b) Convergence in the temperature response of leaf respiration across biomes and plant functional types. *Proceedings of the National Academy of Sciences*, **113**, 3832–3837.
- Hobbs JK, Jiao W, Easter AD, Parker EJ, Schipper LA, Arcus VL (2013) Change in heat capacity for enzyme catalysis determines temperature dependence of enzyme catalyzed rates. *ACS chemical biology*, **8**, 2388–93.
- Hüve K, Bichele I, Rasulov B, Niinemets Ü (2011) When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes



and H<sub>2</sub>O<sub>2</sub> formation. *Plant, Cell & Environment*, **34**, 113–126.

IPCC (2013) *Climate Change 2013: the physical science basis*. Cambridge, UK & New York, NY, USA: Cambridge University Press.

Kruse J, Adams MA (2008) Three parameters comprehensively describe the temperature response of respiratory oxygen reduction. *Plant, Cell & Environment*, **31**, 954–967.

Kruse J, Rennenberg H, Adams MA (2011) Steps towards a mechanistic understanding of respiratory temperature responses. *New Phytologist*, **189**, 659–677.

Lloyd J, Taylor J (1994) On the temperature dependence of soil respiration. *Functional ecology*, **8**, 315–323.

Ma S, Osuna JL, Verfaillie J, Baldocchi DD (2017) Photosynthetic responses to temperature across leaf–canopy–ecosystem scales: a 15-year study in a Californian oak-grass savanna. *Photosynthesis Research*, **132**, 277–291.

Noguchi K, Yamori W, Hikosaka K, Terashima I (2015) Homeostasis of the temperature sensitivity of respiration over a range of growth temperatures indicated by a modified Arrhenius model. *New Phytologist*, **207**, 34–42.

O’Sullivan OS, Weerasinghe KWLK, Evans JR, Egerton JJG, Tjoelker MG, Atkin OK (2013) High-resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*) reveal high-temperature limits to respiratory function. *Plant, cell & environment*, **36**, 1268–84.

O’Sullivan OS, Heskell MA, Reich PB et al. (2017) Thermal limits of leaf metabolism across biomes. *Global Change Biology*, **23**, 209–223.

Ow LF, Griffin KL, Whitehead D, Walcroft AS, Turnbull MH (2008a) Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides* x *nigra*. *New Phytologist*, **178**, 123–134.

Ow LF, Whitehead D, Walcroft AS, Turnbull MH (2008b) Thermal acclimation of respiration but not photosynthesis in *Pinus radiata*. *Functional Plant Biology*, **35**, 448–461.

Prentice I, Farquhar G, Fasham M et al. (2001) The carbon cycle and atmospheric carbon dioxide. In: *Climate Change 2001: the scientific basis. Contribution of Working Group I to the third assessment report of the Intergovernmental Panel on Climate Change* (eds Houghton J, Ding Y, Griggs D, Noguer M, van der Linden P, Dai X, Maskell K, Johnson C), pp. 183–237. Cambridge,

UK: Cambridge University.

Robinson JM, O'Neill TA, Ryburn J, Liang LL, Arcus VL, Schipper LA (2017) Rapid laboratory measurement of the temperature dependence of soil respiration and application to seasonal changes in three diverse soils. *Biochemistry*, **133**, 101–112.

Schipper LA, Hobbs JK, Rutledge S, Arcus VL (2014) Thermodynamic theory explains the temperature optima of soil microbial processes and high Q<sub>10</sub> values at low temperatures. *Global change biology*, **20**, 3578–86.

Schrader SM, Wise RR, Wacholtz WF, Ort DR, Sharkey TD (2004) Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. *Plant, Cell and Environment*, **27**, 725–735.

Skulachev VP (1998) Uncoupling: new approaches to an old problem of bioenergetics. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **1363**, 100–124.

Slot M, Kitajima K (2015) General patterns of acclimation of leaf respiration to elevated temperatures across biomes and plant types. *Oecologia*, **177**, 885–900.

Taylor AE, Giguere AT, Zobelein CM, Myrold DD, Bottomley PJ (2016) Modeling of soil nitrification responses to temperature reveals thermodynamic differences between ammonia-oxidizing activity of archaea and bacteria. *The ISME Journal*, **11**, 896–908.

Thomas TM, Scopes RK (1998) The effects of temperature on the kinetics and stability of mesophilic and thermophilic 3-phosphoglycerate kinases. *The Biochemical journal*, **330**, 1087–95.

Tjoelker MG, Oleksyn J, Reich PB (2001) Modelling respiration of vegetation: evidence for a general temperature-dependent Q<sub>10</sub>. *Global Change Biology*, **7**, 223–230.

Vanderwel MC, Slot M, Lichstein JW et al. (2015) Global convergence in leaf respiration from estimates of thermal acclimation across time and space. *New Phytologist*, **207**, 1026–1037.

Wright IJ, Dong N, Maire V et al. (2017) Global climatic drivers of leaf size. *Science (New York, N.Y.)*, **357**, 917–921.

**Table1** The mean values and standard errors of  $a(\text{MMRT})$ ,  $\Delta H_{T_0}^{\ddagger}$  and  $\Delta C_p^{\ddagger}$ , optimum temperature ( $T_{\text{opt}}$ ) and inflection temperature ( $T_{\text{inf}}$ ) from MMRT across

Biomes and PFTs

| Biomes           | $a(\text{MMRT})$             | $\Delta H_{T_0}^{\ddagger}$<br>(kJ mol <sup>-1</sup> ) | $\Delta C_p^{\ddagger}$<br>(kJ mol <sup>-1</sup> K <sup>-1</sup> ) | $T_{\text{opt}}$ (°C)  | $T_{\text{inf}}$ (°C) | N of fits<br>(species/leaf) | N of $T_{\text{opt}}/T_{\text{inf}}$<br>(species/leaf) |
|------------------|------------------------------|--|--|------------------------|-----------------------|-----------------------------|--|
| Tu               | 0.9194±0.1033 <sup>a</sup>   | 52.3±1.2 <sup>a</sup>                                  | -1.3±0.1 <sup>a</sup>  | 64.0±3.2 <sup>a</sup>  | 38.4±2.0 <sup>a</sup> | 20/79                       | 19/59  |
| BF               | 0.0150±0.0819 <sup>cd</sup>  | 50.2±1.3 <sup>a</sup>                                  | -0.4±0.2 <sup>a</sup>  | 70.1±3.8 <sup>a</sup>  | 42.6±2.3 <sup>a</sup> | 25/96                       | 18/43  |
| TeDF             | 0.2806±0.0995 <sup>de</sup>  | 56.2±2.8 <sup>a</sup>                                  | 0.02±0.3 <sup>a</sup>  | 76.0±5.5 <sup>a</sup>  | 48.3±3.5 <sup>a</sup> | 10/38                       | 4/7  |
| TeW              | 0.2931±0.0571 <sup>bc</sup>  | 56.1±3.0 <sup>a</sup>                                  | -0.3±0.3 <sup>a</sup>  | 64.2±2.2 <sup>a</sup>  | 40.5±1.2 <sup>a</sup> | 67/193                      | 40/81  |
| TeRF             | 0.0107±0.1248 <sup>cd</sup>  | 52.2±2.0 <sup>a</sup>                                  | -0.2±0.2 <sup>a</sup>  | 79.5±4.8 <sup>a</sup>  | 48.6±2.8 <sup>a</sup> | 12/45                       | 7/12   |
| TrRF_lw          | -0.5440±0.0716 <sup>c</sup>  | 53.6±1.3 <sup>a</sup>                                  | -0.3±0.1 <sup>a</sup>  | 65.9±2.0 <sup>a</sup>  | 40.9±1.2 <sup>a</sup> | 81/205                      | 49/75  |
| TrRF_hi          | 0.5252±0.0854 <sup>ab</sup>  | 56.0±3.5 <sup>a</sup>                                  | -0.7±0.2 <sup>a</sup>  | 70.7±4.5 <sup>a</sup>  | 42.8±3.9 <sup>a</sup> | 16/17                       | 10/10  |
| PFTs             |                              |  |  |                        |                       |                             |  |
| C <sub>3</sub> H | 0.7453±0.1352 <sup>a</sup>   | 52.3±1.2 <sup>a</sup>                                  | -1.4±0.2 <sup>a</sup>  | 67.7±3.5 <sup>a</sup>  | 40.1±2.3 <sup>a</sup> | 13/50                       | 13/43  |
| SEv              | 0.3130±0.1042 <sup>ab</sup>  | 50.2±1.3 <sup>a</sup>                                  | -0.5±0.4 <sup>a</sup>  | 61.3±2.6 <sup>a</sup>  | 38.7±1.3 <sup>a</sup> | 35/104                      | 24/47  |
| NIEv             | 0.3745±0.1434 <sup>ab</sup>  | 56.2±2.8 <sup>a</sup>                                  | -0.6±0.2 <sup>a</sup>  | 72.6±3.9 <sup>a</sup>  | 45.3±2.5 <sup>a</sup> | 13/48                       | 8/19   |
| BlDcTmp          | -0.0460±0.0819 <sup>bc</sup> | 56.1±3.0 <sup>a</sup>                                  | -0.5±0.2 <sup>a</sup>  | 67.7±3.0 <sup>a</sup>  | 41.7±1.8 <sup>a</sup> | 40/150                      | 28/66  |
| BlEvTmp          | 0.2907±0.0828 <sup>bc</sup>  | 52.2±2.0 <sup>a</sup>                                  | 0.1±0.4 <sup>a</sup>   | 70.9±4.3 <sup>a</sup>  | 44.2±2.5 <sup>a</sup> | 34/104                      | 16/28  |
| BlEvTrp          | -0.3523±0.0744 <sup>c</sup>  | 53.6±1.3 <sup>a</sup>                                  | -0.4±0.1 <sup>a</sup>  | 67.2±1.9 <sup>a</sup>  | 41.4±1.3 <sup>a</sup> | 93/207                      | 56/82  |
| BlDcTrp          | -0.3299±0.2802 <sup>bc</sup> | 56.0±3.5 <sup>a</sup>                                  | -0.7±0.5 <sup>a</sup>  | 59.8±10.0 <sup>a</sup> | 39.5±2.9 <sup>a</sup> | 3/10                        | 2/2  |
| Global Mean      | -0.0008±0.0460               | 54.0±1.0   | -0.4±0.1   | 67.0±1.2               | 41.4±0.7              |                             |  |

Tu: Tundra, BE: Boreal Forest, TeDF: Temperature Deciduous Forest, TeW: Temperature Woodland, TeRF: Temperature Rainforest, TrRF\_lw: Tropical Rainforest at low elevation, TrRF\_hi: Tropical Rainforest at high elevation. C<sub>3</sub>H: C3 Herbaceous, SEv: Evergreen Shrubs, NIEv: Needle-leaf evergreen, BIDcTmp: Broadleaf Deciduous Temperate, BIEvTmp: Broadleaf Evergreen Temperate, BIEvTrp: Broadleaf Evergreen Tropical, BIDcTrp: Broadleaf Deciduous Tropical

Within columns, values with the same letter were not significantly different for the pairwise comparison across Biomes and PFTs. N of fits (species/leaf) is the initial sample size of the data for fitting MMRT, species/leaf denotes the number of species or leaf samples in each biome and PFT. N of T<sub>opt</sub>/T<sub>inf</sub> denotes the number of credible fits in calculating the T<sub>opt</sub> and T<sub>inf</sub> within biological meaningful range.

**Fig. 1** The correspondence between MMRT and LP function in describing the temperature response of leaf respiration. The black and grey squares are the predicted optimum temperature ( $T_{opt}$ ) from MMRT (62.32 °C) and LP ( 62.07 °C) functions respectively. The black and grey circles are the inflection temperature ( $T_{inf}$ ), 34.40 and 37.25°C from MMRT and LP, respectively.  $T_{opt}$  and  $T_{inf}$  in MMRT and LP are mainly defined by the curvature terms from MMRT and LP, i.e.,  $\Delta C_p^\ddagger$  and  $c$ , respectively. The  $T_{opt}$  and  $T_{inf}$  could varies between 25 and 100 °C depending on the magnitude of negative curvature terms.

**Fig. 2** Comparisons of predictive power of MMRT, LP (Log-Polynomial) and Arrhenius models, showing both MMRT and LP models are equivalent and more powerful than the Arrhenius model in characterizing the temperature response of  $R$ . (a) shows the capability of three models in characterising the temperature response of measured leaf respiration using the  $R$ - $T$  curve of species *Anemone narcissiflora* in tundra (b) shows the corresponding residuals from MMRT (solid circles), LP (open circles line) and Arrhenius (open squares) models from panel (a). (c) compares AICc values across the three models.

**Fig. 3** Comparisons between fitted parameters derived from MMRT and polynomial following Eq.7. All the parameters between polynomial and MMRT are very tightly correlated.

**Fig. 4** (a) The relationship between  $a$ (MMRT) and aridity index (the ratio between mean annual precipitation (MAP) and potential evapotranspiration (PET)) across 18 sites covering different climates. The black dash line is a linear regression fit ( $y=-0.64x+ 0.59$ ). The colour demonstrates the mean annual temperature (MAT) for each of the sites and the symbol size increases with the mean annual precipitation (MAP). (b) shows the temperature response curve from MMRT at two sites with contrasting environments. The solid line indicates the temperature response of leaf respiration at Toolik, Alaska, US using the mean parameters of MMRT retrieved from 79 individual lnR- $T$  curves. The dash line represents the mean temperature response of plant leaves at Canberra, Australia using

the retrieved parameters from 15 individual lnR-T curves. The vertical dish line indicates the reference temperature,  $T_0$  (298.15K, 25°C). The grey bands show the  $R$  between MAT and mean temperature in the warmest quarter (TWQ) of the year. The similar magnitude of  $R$  from two sites suggests a homeostasis of respiration in plants that maintains a comparable  $R$  under the growth temperature of their habitats.



