

# Thermal acclimation of leaf photosynthetic traits in an evergreen woodland, consistent with the coordination hypothesis

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15 **Abstract.** Ecosystem models commonly assume that key photosynthetic traits, such as carboxylation capacity measured at a standard temperature, are constant in time. The temperature responses of modelled photosynthetic or respiratory rates then depend entirely on enzyme kinetics. Optimality considerations however suggest this assumption may be incorrect. The ‘coordination hypothesis’ (that Rubisco- and electron-transport limited rates of photosynthesis are co-limiting under typical daytime conditions) predicts, instead, that carboxylation ( $V_{cmax}$ ) capacity should acclimate so that it increases somewhat with growth temperature – but less steeply than its instantaneous response, implying that  $V_{cmax}$  when normalized to a standard temperature (e.g. 25°C) should *decline* with growth temperature. With additional assumptions, similar predictions can be made for electron-transport capacity ( $J_{max}$ ) and mitochondrial respiration in the dark ( $R_{dark}$ ). To explore these hypotheses, photosynthetic measurements were carried out on woody species during the warm and the cool seasons in the semi-arid Great Western Woodlands, Australia, under broadly similar light environments. A consistent proportionality between  $V_{cmax}$  and  $J_{max}$  was found across species.  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  increased with temperature in most species, but their values standardized to 25°C declined. The  $c_i:c_a$  ratio increased slightly with temperature. The leaf N:P ratio was lower in the warm season. The slopes of the relationships of log-transformed  $V_{cmax}$  and  $J_{max}$  to temperature were close to values predicted by the coordination hypothesis, but shallower than those predicted by enzyme kinetics.

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## 30 **1 Introduction**

Net photosynthetic CO<sub>2</sub> uptake ( $A_{net}$ ) depends on temperature in all vegetation models, but models commonly disregard possible acclimation of the parameters determining  $A_{net}$  to temporal variations in the growth environment. There are plentiful

data on the instantaneous (minutes to hours) temperature responses of photosynthetic uptake (Hikosaka et al., 2006; Sage and Kubien, 2007; Way and Sage, 2008), but data on the responses of photosynthetic traits on ecologically relevant time scales (days to years) are scarce (Wilson et al., 2000; Lin et al., 2013). Nonetheless there is evidence that temperature responses of biochemical processes are a function of plant growth temperature, and not just instantaneous temperature: from studies comparing species (Miyazawa and Kikuzawa, 2006; Kattge and Knorr, 2007), and experiments or measurements on single species (Medlyn et al., 2002b; Onoda et al., 2005). Neglecting acclimation to the growth temperature could lead to incorrect model estimates of the responses of primary production and ecosystem carbon budgets to climate change.

Farquhar et al. (1980) provided the standard model to predict photosynthetic responses to environment in  $C_3$  plants. The model describes photosynthesis as instantaneously determined by the slower of two biochemical rates: the carboxylation of *RuBP* (ribulose-1,5-bisphosphate), dependent on Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity ( $V_{cmax}$ ); and electron transport for *RuBP* regeneration in the Calvin cycle, which is dependent on light intensity and (at high light intensity) limited by the capacity of the electron transport chain ( $J_{max}$ ). Both rates are influenced by intercellular  $CO_2$  concentration ( $c_i$ ), which in turn is partially regulated by stomatal conductance ( $g_s$ ). Unlimited mesophyll conductance (Miyazawa and Kikuzawa, 2006; Lin et al., 2013) remains the commonest implementation of the Farquhar model; ‘apparent’  $V_{cmax}$  and  $J_{max}$  values can be calculated from  $A-c_i$  curves under this assumption provided  $K_C$  and  $K_O$  (the Michaelis-Menten coefficients for carboxylation and oxygenation, respectively) are appropriately specified.  $V_{cmax}$  and  $J_{max}$  are highly variable both within and between species (Wullschlegel, 1993), ranging through two to three orders of magnitude. Despite the accepted importance of these parameters for predicting rates of net  $CO_2$  exchange in natural ecosystems, a full understanding of how changes in the environment affect these quantities is lacking.

The ‘coordination hypothesis’ (Chen et al., 1993; Field and Mooney, 1986; Maire et al., 2012) predicts that values of  $V_{cmax}$  and  $J_{max}$  should acclimate, in time as well as in space, in such a way that carboxylation and *RuBP* regeneration are co-limiting under average daytime conditions. This has been suggested often in the literature, both as a prediction based on optimality considerations (e.g. Von Caemmerer and Farquhar, 1981; Haxeltine and Prentice, 1996; Dewar, 1996) and as an empirical observation, consistent with the finding that carboxylation is limiting at saturating light intensity – as has been shown to apply in the great majority of cases (De Kauwe et al., 2015). The coordination hypothesis makes a number of testable predictions regarding photosynthetic properties of woody plants experiencing large seasonal variations in growth temperature, including an increase in  $V_{cmax}$  with rising growth temperature – because at higher daily temperatures, somewhat greater Rubisco activity is required to match any given rate of photosynthesis. On the other hand, it predicts that the value of  $V_{cmax}$  normalized to 25°C – and with it, the leaf N per unit area (Maire et al., 2012; Dong et al., 2017) – should decline with increasing daily average temperature, because the quantities of proteins needed to maintain a given level of photosynthetic activity decline more steeply with temperature than does the predicted increase in  $V_{cmax}$ . These predictions were strongly supported by experiments on tree species grown at different temperatures (Scafero et al., 2017). Scafero et al. (2017) also

demonstrated that the acclimation of  $V_{\text{cmax}}$  involves changes in Rubisco amount, and the relative allocation of leaf N to Rubisco. If it is further assumed that  $J_{\text{max}}$  covaries closely with  $V_{\text{cmax}}$ , as is commonly observed, then similar predictions apply to the acclimation of  $J_{\text{max}}$ . Wang et al. (2017) have provided a fuller treatment of  $J_{\text{max}}$  acclimation, based on a cost-minimizing principle that also correctly predicts the dependence of the ratio  $J_{\text{max}}/V_{\text{cmax}}$  on growth temperature (Kattge and Knorr, 2007; Wang et al., 2017). Note that the coordination hypotheses as presented here is the "strong form" of the hypothesis, which allows the metabolic component of leaf N to be optimized – thus differing from an alternative interpretation, in which leaf N remains constant and only the  $J_{\text{max}}/V_{\text{cmax}}$  ratio is allowed to vary (Medlyn 1996; Ali et al. 2015; Quebbeman and Ramirez 2016).

$R_{\text{dark}}$  (leaf respiratory  $\text{CO}_2$  release in darkness) is known to acclimate to temperature, although little is known about how this might be linked to the coordination hypothesis. Acclimation results in  $R_{\text{dark}}$  being of similar magnitude in plants grown at different temperatures, when measured at their respective growth temperatures (Larigauderie and Körner, 1995; Atkin and Tjoelker, 2003) and also results in  $R_{\text{dark}}$  at  $25^\circ\text{C}$  increasing upon cold acclimation and declining upon acclimation to warmer temperature (Reich et al., 2016). Growth temperature-dependent changes in  $R_{\text{dark}}$  at a standard temperature can occur over periods of 1-3 days (Atkin et al., 2000; Bolstad et al., 2003; Lee et al., 2005; Zaragoza-Castells et al., 2007; Armstrong et al., 2008). A data synthesis of global patterns in  $R_{\text{dark}}$  (Atkin et al., 2015) showed that geographic variation in  $R_{\text{dark}}$  at growth temperature from the tropics to the tundra is much smaller than would be expected on the basis of enzyme kinetics. In that study, leaf  $R_{\text{dark}}$  at standard temperature was shown to increase with decreasing growth temperature at a global scale (Atkin et al., 2015). Observed global patterns in  $R_{\text{dark}}$  are consistent with the acclimation of  $R_{\text{dark}}$  to global patterns in growth temperature (Slot and Kitajima, 2015; Vanderwel et al., 2015).

Many ecosystem models assume optimality criteria for stomatal behaviour, in which carbon assimilation is traded off against water loss. Prentice et al. (2014) provided field evidence supporting the 'least-cost' hypothesis, stating that plants adopt an optimal  $c_i:c_a$  ratio that minimizes the combined costs per unit carbon assimilation of maintaining the capacities for carboxylation ( $V_{\text{cmax}}$ ) and water transport. This hypothesis predicts that the  $c_i:c_a$  ratio should increase with temperature, due to lower water viscosity (reducing water costs) and higher photorespiration (increasing carboxylation costs) (Prentice et al., 2014), while declining with vapour pressure deficit (VPD), due to increasing water costs (Prentice et al., 2011).

In the current study, we present leaf-level measurements carried out during the warm and the cool seasons in the semi-arid environment of the Great Western Woodlands of southwestern Australia. By sampling during both seasons, we were able to compare observations under broadly overlapping light conditions but at contrasting temperatures. We are not aware of any previous study that has tested whether seasonal temperature acclimation is consistent with the coordination hypothesis. We explore the idea by comparing the field-observed relationships of each trait to temperature with the theoretical acclimation of photosynthetic traits (as predicted by the coordination hypothesis) and with the alternative, i.e. the relationship of each trait to temperature to be expected if it were controlled only by enzyme kinetics (i.e. no acclimation).

## 2 Materials and Methods

### 2.1 Site

Eight representative woody species were studied at the Great Western Woodlands SuperSite (17°07'S, 145°37'E) approximately 70 km north-west of Kalgoorlie, Western Australia. The area has a semi-arid climate and the vegetation is a well-preserved mosaic of temperate woodland, shrubland and mallee. The mean annual precipitation (MAP) during 1970-2013 was 380 mm (Hutchinson, 2014c). The average precipitation is slightly higher during summer months, but this rain often falls during short periods as intense storms. Mean annual temperature (MAT) is 20°C (Hutchinson, 2014a, b). Mean monthly daily temperature minima range between 6°C and 18°C and maxima between 17°C and 35°C. The area is not prone to large shifts in temperature or vpd within days (e.g. cold fronts). Data for daily temperature and shortwave radiation were obtained from the flux tower (TERN Ozflux, [www.ozflux.org.au](http://www.ozflux.org.au)). All trees were sampled within a 5 km radius from the tower. The species studied were the angiosperm trees *Eucalyptus clelandii*, *E. salmonophloia*, *E. salubris* and *E. transcontinentalis* and the shrub *Eremophila scoparia*; the nitrogen-fixing leguminous tree *Acacia aneura* and shrub *A. hemiteles*; and one gymnosperm tree, *Callitris columellaris*. All are evergreen.

### 2.2 Gas exchange measurements

We measured 109  $A-c_i$  curves altogether during the warm season (late March/early April) and the cool season (late August/early September). The same individual plants were sampled in both seasons. A portable infrared gas analyser system (LI-6400; Li-Cor, Inc., Lincoln, NB, USA) was used. Sunlit terminal branches from the top one-third of the canopy were collected and immediately re-cut under water (Domingues et al., 2010). One of the youngest fully expanded leaves, attached to the cut branch, was placed in the leaf chamber. Measurements in the field were taken with the chamber block temperature close to the air ambient temperature. The  $\text{CO}_2$  partial pressure in the chamber for the  $A-c_i$  curves proceeded stepwise down from 400 to 35, back to 400 and then up to 2000  $\mu\text{mol mol}^{-1}$ . Prior to the measurements, we tested plants to determine appropriate light-saturation levels. The photosynthetic photon flux density (PPFD) adopted for measurement ranged between 1500 and 1800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After measuring the  $A-c_i$  curves over about 35 minutes, light was set to zero for five minutes before measuring  $R_{\text{dark}}$ . This was a time-saving compromise to allow four or five replicate curves per machine per day, based on our experience that stable results are commonly obtained after 5 minutes. Moreover, this quick estimate should still be superior to the common practice of deriving  $R_{\text{dark}}$  as one of the parameters in a curve-fitting routine. Following Domingues et al. (2010), we discarded 23  $A-c_i$  curves in which stomatal conductance declined to very low levels – resulting in 86 curves being used in further analyses. TPU (triose phosphate utilization) limitation (Sharkey et al., 2007) was not considered, as it would be unlikely to occur at our field temperatures of above 17 °C.

In theory optimal growth temperature ( $T_{\text{opt}}$ ) could affect the calculation of  $V_{\text{cmax}}$  and  $J_{\text{max}}$ . However, Medlyn et al. (2002b) and Katge and Knorr (2007) have found very good correlations between  $T_{\text{opt}}$  and mean daily temperature; and we

measured  $A-c_i$  curves between morning and early afternoon, avoiding the hottest part of the day. Therefore, it is unlikely that any measurements were carried out above the optimum temperature.

The primary data are available via the TERN Supersites Data Portal (Prober et al., 2015). Reported ratios of  $c_i:c_a$  relate to chamber conditions, with ambient  $\text{CO}_2 \approx 400 \mu\text{mol mol}^{-1}$ .

### 5 2.3 Photosynthetic parameters and their temperature responses

Apparent values of  $V_{c_{\max}}$  and  $J_{\max}$  were fitted using the Farquhar et al. (1980) model. Values were standardized to 25°C ( $V_{c_{\max}25}$  and  $J_{\max25}$ ) using the *in vivo* temperature dependencies given in Bernacchi et al. (2001) and Bernacchi et al. (2003). Following Bernacchi et al. (2009), we used the Arrhenius equation to describe the kinetic temperature responses of  $V_{c_{\max}}$  and  $J_{\max}$ :

$$10 \quad \text{param}(T) = \text{param}(T_{\text{ref}}) \exp \left[ \frac{\Delta H_a}{R} \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T} \right) \right] \quad (1)$$

where  $\Delta H_a$  is the activation energy ( $\text{J mol}^{-1}$ ),  $R$  is the universal gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ), and  $T_{\text{ref}}$  is a reference temperature;  $T$  is the leaf temperature (K). To derive  $R_{\text{dark}25}$  we applied a temperature-dependent  $Q_{10}$  (fractional change in respiration with a 10°C increase in temperature) equation in which  $Q_{10}$  declines with increasing leaf temperature (Atkin and Tjoelker, 2003):

$$15 \quad R_{\text{dark}25} = R_{\text{dark}} \left( 3.09 - 0.043 \left[ \frac{T_{\text{Cref}} + T_C}{2} \right] \right)^{\left[ \frac{T_{\text{Cref}} - T_C}{10} \right]} \quad (2)$$

where 3.09 and 0.04 are empirical constants,  $T_{\text{Cref}} = 25^\circ\text{C}$  and  $T_C$  is leaf temperature ( $^\circ\text{C}$ ).

### 2.4 Nutrient analyses

After completion of each  $A-c_i$  curve, leaves were retained to determine leaf area, dry mass, and mass-based nitrogen (N) and phosphorus (P) concentrations ( $\text{mg g}^{-1}$ ). Leaves were sealed in plastic bags containing moist tissue paper to prevent wilting.

- 20 Leaf area was determined using a 600 dots/inch flatbed top-illuminated optical scanner and Image J software (<http://imagej.nih.gov/ij/>). Leaves were dried in a portable desiccator for 48 hours, to be preserved until the end of the campaign. Subsequently, in the laboratory, leaves were oven-dried for 24 hours at 70°C and the dry weight determined (Mettler-Toledo Ltd, Port Melbourne, Victoria, Australia). Leaf mass per unit area (LMA;  $\text{g m}^{-2}$ ) was calculated from leaf area and dry mass.  $N_{\text{mass}}$  and  $P_{\text{mass}}$  were obtained by Kjeldahl acid digestion of the same leaves (Allen et al., 1974). The leaf material was digested using sulphuric acid 98% and hydrogen peroxide 30%. Digested material was analyzed for N and P
- 25 using a flow injection analyser system (LaChat QuikChem 8500 Series 2, Lachat Instruments, Milwaukee, WI, USA). Area-based N and P values ( $N_{\text{area}}$ ,  $P_{\text{area}}$ ,  $\text{mg m}^{-2}$ ) were calculated as the products of LMA and  $N_{\text{mass}}$  or  $P_{\text{mass}}$ .

## 2.5 Statistical analyses

All statistics were performed in R (R Core Team, 2012). For graphing we used the `ggplot2` package (Wickham, 2010).  $V_{\text{cmax}}$ ,  $V_{\text{cmax}25}$ ,  $J_{\text{max}}$ ,  $J_{\text{max}25}$ ,  $R_{\text{dark}}$ ,  $R_{\text{dark}25}$ ,  $N_{\text{area}}$  and  $P_{\text{area}}$  data were natural log-transformed to approximate a normal distribution of values. Log transformation of the photosynthetic parameters has the added advantage that the slopes of log-transformed values with respect to temperature are expected to be approximately linear, due to the exponential nature of biochemical temperature dependencies generally. The ratio  $c_i:c_a$  however was not transformed because of its small variance, and approximately normal distribution, in this study. Linear regression (`lm`) was used to test dependencies among parameters. Slopes and elevations of regressions were compared using standardized major axis regression with the `smatr` package (Warton et al., 2006). The Welch two-sample  $t$ -test was used to test pairwise differences in traits (e.g. differences between the warm season and the cool season measurements). Generalized linear models (`glm`) were used to test acclimation to temperature across species, with temperature as the single quantitative predictor and species as a factor.

## 2.6 Comparison of observed and predicted responses to temperature

Regression slopes of each natural log-transformed variable to temperature (obtained using species as a factor, to control for differences in the magnitudes of values for different species) were compared with theoretically derived values based on alternative hypotheses: (a) based on enzyme kinetics (without acclimation), and (b) based on the coordination hypothesis for  $V_{\text{cmax}}$  (Fig. 1),  $J_{\text{max}}$  and  $R_{\text{dark}}$  and the least-cost hypothesis for  $c_i:c_a$ . ‘Kinetic’ temperature responses were predicted from equation (1), which can also be written:

$$\ln \text{param}(T) - \ln \text{param}(T_{\text{ref}}) = (\Delta H_a/R) (1/T_{\text{ref}} - 1/T) \quad (3)$$

which in turn is well approximated by linearizing around  $T_{\text{ref}}$ :

$$\ln \text{param}(T) - \ln \text{param}(T_{\text{ref}}) \approx (\Delta H_a/R) (1/T_{\text{ref}}^2) \Delta T \quad (4)$$

where  $\Delta T = T - T_{\text{ref}}$ . Thus, the ‘kinetic’ slopes of  $\ln \text{param}(T)$  versus  $T$  can be predicted from Eq. (4) and compared directly with the fitted slopes obtained with a generalized linear model. We set  $T_{\text{ref}} = 298$  K (which is both conventional, and close to the median measurement temperature in our data set). We applied  $\Delta H_a$  values based on *in vivo* measurement at 25°C by Bernacchi et al. (2001) for  $V_{\text{cmax}}$  (65 330 J mol<sup>-1</sup> K<sup>-1</sup>). For  $J_{\text{max}}$ , we used the  $\Delta H_a$  value based on *in vivo* measurements by Bernacchi et al. (2003) on plants that had been grown at 25°C (43 900 J mol<sup>-1</sup> K<sup>-1</sup>). For  $R_{\text{dark}}$ , we used a value of  $\Delta H_a$  (50230 J mol<sup>-1</sup> K<sup>-1</sup>) calculated from Eq. (2). Although  $\Delta H_a$  values can vary among species and with temperature (Von Caemmerer, 2000), for simplicity we adopted the same  $\Delta H_a$  for all species and temperatures. This approximation could affect interspecies comparisons of  $\Delta H_a$ -dependent parameters, but should not interfere with the comparison of theoretical and fitted slopes.

The ‘acclimated’ response of  $V_{\text{cmax}}$  according to the coordination hypothesis was predicted by setting the Rubisco- and electron-transport limited rates of photosynthesis to be equal. To simplify matters, we disregarded the curvature of the response of electron transport to PPFD at values below the point of Rubisco limitation, giving:

$$V_{\text{cmax}} = \varphi_0 I_{\text{abs}} (c_i + K)/(c_i + 2\Gamma^*) \quad (5)$$

- 5 where  $\varphi_0$  is the intrinsic quantum efficiency of photosynthesis,  $I_{\text{abs}}$  is the absorbed PPFD,  $K$  is the effective Michaelis-Menten coefficient for carbon fixation and  $\Gamma^*$  is the photorespiratory compensation point. The theoretical (acclimated) sensitivity of  $V_{\text{cmax}}$  to temperature was then calculated from the derivative of equation (5):

$$\partial \ln V_{\text{cmax}}/\partial T = (1/V_{\text{cmax}}) \partial V_{\text{cmax}}/\partial T = (\partial c_i/\partial T) [1/(c_i + K) - 1/(c_i + 2\Gamma^*)] + (\partial K/\partial T) [1/(c_i + K)] - 2 (\partial \Gamma^*/\partial T) [1/(c_i + 2\Gamma^*)] \quad (6)$$

- 10 We evaluated Eq. (6) at  $T = 298$  K and  $c_i = 200 \mu\text{mol mol}^{-1}$  (approximately the median of our observed values of  $c_i$ ), using the temperature dependencies of  $K$  and  $\Gamma^*$  from Bernacchi et al. (2001). The temperature dependency of  $K$  was determined from those of the constituent terms  $K_C$  and  $K_O$  (the Michaelis-Menten coefficients for carboxylation and oxygenation, respectively) (Wang et al., 2017). We estimated the theoretical acclimated slope for  $J_{\text{max}}$  as being the acclimated slope of  $V_{\text{cmax}}$ , minus the difference between the kinetic slopes of  $V_{\text{cmax}}$  and  $J_{\text{max}}$ ; this is equivalent to assuming that the ratio of  $J_{\text{max}}$  to  $V_{\text{cmax}}$  at  $25^\circ\text{C}$  is constant. We further assumed that  $R_{\text{dark}}$  (on acclimation) should be an invariant fraction of  $V_{\text{cmax}}$ , implying the same acclimated temperature response for  $R_{\text{dark}}$  as for  $V_{\text{cmax}}$ .

The least-cost hypothesis (Prentice et al., 2014) provides an optimal value for  $c_i:c_a$ , denoted by  $\chi_o$ , such that:

$$\chi^* = \chi_o/(1 - \chi_o) = \sqrt{(bK/1.6aD)} \quad (7)$$

- where  $b$  is the (assumed constant) ratio of  $R_{\text{dark}}$  to  $V_{\text{cmax}}$  and  $a$  is the cost of maintaining the water-transport pathway. Both  $K$  and  $a$  are temperature-dependent ( $a$  because it is proportional to the viscosity of water,  $\eta$ ). Holding vapour pressure deficit ( $D$ ) constant, we obtained an expression for  $\partial \chi^*/\partial T$ :

$$\partial \chi^*/\partial T = (\chi^*/2) (\partial \ln K/\partial T - \partial a/\partial T) \quad (8)$$

and from Eqs. (7) and (8),

$$\partial \chi_o/\partial T = (\chi_o/2) (1 - \chi_o) (\partial \ln K/\partial T - \partial a/\partial T) \quad (9)$$

- 25 We evaluated Eq. (9) at  $T = 298$  K,  $\chi_o = 0.5$  and  $c_a = 400$  ppm using the known temperature dependencies of  $K$  and  $\eta$ .

### 3 Results

#### 3.1 Relationships among photosynthetic parameters

When measured at near-ambient air temperature, species-average  $V_{cmax}$  values ranged across seasons from 44.4 to 105  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $J_{max}$  from 77.4 to 160  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $R_{dark}$  from 1.16 to 3.14  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and  $c_i:c_a$  from 0.39 to 0.60 (at ambient  $\text{CO}_2 \approx 400 \mu\text{mol mol}^{-1}$ ). At the prevailing air temperatures,  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  were systematically higher in the warm season, while their values standardised to 25°C were higher in the cool season (Fig. 2). The  $c_i:c_a$  ratio also exhibited significantly higher average values in the warm season in six out of eight species (not shown).

$V_{cmax}$  and  $J_{max}$  were closely correlated across species within and across seasons (Fig. 3). The warm and the cool season regression equations relating  $V_{cmax}$  and  $J_{max}$  were significant, and statistically indistinguishable. The warm and the cool season slopes of regressions forced through the origin are shown in Fig. 3. (We show regressions through the origin here because the intercept (a) is extrapolated well beyond the range of the data, and (b) has no clear biological meaning.) Regressions between  $V_{cmax}$  and  $J_{max}$  for the warm and the cool seasons together yielded  $J_{max} = 0.84 V_{cmax} + 55.2$  ( $p < 0.05$ , slope standard error =  $\pm 0.2$ ). There were positive correlations between  $R_{dark25}$  and both  $V_{cmax}$  and  $J_{max}$  across seasons, as well as strong negative correlations between species-average  $c_i:c_a$  ratios and both  $V_{cmax}$  and  $J_{max}$  across seasons (Fig. 4). Log transformed  $R_{dark}$  was only correlated to  $V_{cmax}$  and  $J_{max}$  ( $p < 0.05$ ) for the individual species *E. salmonophloia* and *C. columellaris*.

#### 3.2 Leaf gas exchange trait responses to temperature

Based on data from all species together,  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  increased with leaf temperature, while the corresponding normalized (to 25°C) values declined with leaf temperature ( $p < 0.05$ , Fig. 5). The  $c_i:c_a$  ratio also increased slightly but significantly with leaf temperature, ( $p < 0.05$ ; Fig. 6a). The ratio  $J_{max}:V_{cmax}$  did not correlate with temperature based on the data from all species together, but it was negatively correlated with temperature for *E. salmonophloia*, *E. scoparia* and *C. columellaris* (not shown). Excluding the two N-fixing species, and/or the one gymnosperm, from the dataset had no effect on these results.

The relationship between ambient air temperature ( $T_{air}$ , °C) and leaf temperature ( $T_{leaf}$ , °C) as measured in the gas-exchange system was  $T_{leaf} = 1.01 T_{air} + 0.35$  ( $p < 0.05$ ,  $R^2 = 0.96$ ). Regression slopes between photosynthetic parameters and  $T_{air}$  showed no significant differences from those calculated using  $T_{leaf}$ , but the goodness of fit was weaker with  $T_{air}$  than with  $T_{leaf}$ . We also fitted regressions using  $T_{day}$  (the daily mean temperature). Again the slopes did not change, but the goodness of fit was further reduced. The factor 'season' (included as a predictor in a generalized linear model, in addition to  $T_{leaf}$ ) did not improve model fit.



Within individual species, we also found positive responses of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  to temperature, and negative responses when the parameters were standardised to 25°C (Fig. 7). The response of the  $c_i:c_a$  ratio to leaf temperature was similar in most species (Fig. 6b). Within-species responses of  $R_{\text{dark}}$  to leaf temperature were weaker and less consistent (Fig. 7), suggesting that respiration had acclimated to a greater extent than was the case for  $V_{\text{cmax}}$  and  $J_{\text{max}}$ .

5 The average VPD value during the warm season was 1.13 kPa, and during the cool season 0.45 kPa. The average leaf-to-air VPD (i.e. corrected to leaf temperature) during the warm season was 2.5 kPa, and during the cool season 1.44 kPa. However, there was very little difference in stomatal conductance at light saturation: 0.064 – 0.082 – 0.101 (lower quartile – median – upper quartile) mol m<sup>-2</sup> s<sup>-1</sup> for the warm season, and 0.057 – 0.078 – 0.085 mol m<sup>-2</sup> s<sup>-1</sup> for the cool season.

10 Incoming shortwave radiation at the surface was used as a proxy for PPFD. Daily values ranged from 90 to 256 W m<sup>-2</sup> and averaged 193 W m<sup>-2</sup>. Averages for the warm and the cool seasons sampling periods were not significantly different. None of the photosynthetic parameters showed any relationship with shortwave radiation.

### 3.3 Leaf N and P relationships to photosynthetic traits and temperature

Area-based rates of leaf gas exchange traits were not systematically related to total leaf  $N_{\text{area}}$  or  $P_{\text{area}}$  (not shown). There was a positive relationship between N and P (by mass) taking all species together, and within three of the species ( $p < 0.05$ ; Fig. 15 8). High values of foliar N:P ratios (> 16) in seven out of eight species (Fig. 9) may suggest that P in this ecosystem is more limiting to growth than N (Westoby and Wright, 2006). N:P ratios declined with increasing temperature ( $p < 0.05$ ). *A. aneura* and *A. hemiteles* presented the highest N:P, as expected for N-fixing species.

We investigated how the ratio of  $V_{\text{cmax}}$  (and  $V_{\text{cmax}25}$ ) to  $N_{\text{area}}$  varied with ambient temperature. The results were generally very similar to the analysis of  $V_{\text{cmax}}$  (and  $V_{\text{cmax}25}$ ) to temperature (Figs. 5 and 7), but they were significant ( $p < 0.05$ ) 20 for seven out of eight species (all except the gymnosperm *Callitris columellaris*) for both  $V_{\text{cmax}}$  and  $V_{\text{cmax}25}$  (not shown).

### 3.4 Quantitative temperature responses

For  $V_{\text{cmax}}$  and  $J_{\text{max}}$ , the fitted slopes with leaf temperature were shallower than the predicted ‘kinetic’ slopes by a margin that greatly exceeded their 95% confidence limits (Table 1). The ‘kinetic’ values are what would be expected if the activities of the relevant enzyme complexes remained constant with changing growth temperature. The coordination hypothesis predicts 25 much shallower ‘acclimated’ rate-temperature slopes (Fig. 1). The acclimated slope for  $V_{\text{cmax}}$  can be predicted from the temperature dependencies of  $K$  and the photorespiratory compensation point ( $I^*$ ). The acclimated slope for  $V_{\text{cmax}}$  falls just marginally above the 95% confidence interval for the fitted slope. The acclimated slope of  $J_{\text{max}}$  falls well within the 95% confidence interval for the fitted slope. For  $R_{\text{dark}}$  the acclimated and kinetic slopes are closer together, and both fall within the 95% confidence interval of the fitted slope.

There is no 'kinetic' response of  $c_i:c_a$ , but the least-cost hypothesis predicts a positive response to temperature. This was observed, although the fitted slope of the response to temperature was shallower than predicted.

## 4 Discussion

### 5 4.1 Quantitative ranges of photosynthesis traits

Values of  $V_{cmax}$ ,  $J_{max}$ ,  $R_{dark}$  and  $c_i:c_a$  for the eight species measured here were within ranges commonly reported.  $V_{cmax}$  and  $J_{max}$  were generally lower than expected for desert species ( $153 \pm 54 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), but higher than typical values for mesic perennial species ( $53 \pm 15 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Wullschleger, 1993). The values were also high compared with trees from savannas with twice the annual precipitation (range  $20 - 90 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) (Domingues et al., 2010). The  $c_i:c_a$  ratios fall within the range typical for dry environments (Prentice et al., 2014).

### 4.2 Comparison between seasons

Our results were consistent with acclimation of photosynthetic traits to temperature as predicted by the coordination hypothesis. When measured at the prevailing ambient temperature,  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  were all generally higher in the warm season than in the cool season, whereas values standardized to  $25^\circ\text{C}$  were generally lower in the warm season than in the cool season (Fig. 2). This is *prima facie* evidence for active seasonal acclimation, in agreement with the coordination hypothesis that predicts lower allocation of N to Rubisco and other photosynthetic enzymes at higher temperatures, offsetting the increase in enzyme activity with elevated temperature. Moreover, absence of acclimation (as found in Way and Yamori, 2014) should result in no relationship between  $V_{cmax25}$  and leaf temperature, while the negative relationship found here is evidence for acclimation (Figs. 5 and 7).

The comparison of fitted and theoretically predicted slopes (Table 1) reveals not only that the responses of  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  to ambient temperature were smaller than would be predicted from enzyme kinetics alone, but also that the observed responses were close to, or (in the case of  $J_{max}$ ) statistically indistinguishable from, the responses predicted by the coordination hypothesis. The response of  $c_i:c_a$  is in the same direction (positive) as the response predicted by the least-cost hypothesis, but only about half as large, probably due to the opposing effect (reduction in  $c_i:c_a$ ) of larger VPD in the warm season than in the cool season (Prentice et al., 2014).

$V_{cmax}$  and  $J_{max}$  were strongly and positively correlated across species (Fig. 3), and the relationship did not shift significantly between seasons. Some studies have reported a lower  $J_{max}:V_{cmax}$  ratio in warmer seasons compared to cooler seasons (Medlyn et al., 2002a; Lin et al., 2013). Our data show a tendency in this direction, but not enough to be significant

(Fig. 3).  $V_{\text{cmax}}$  and  $J_{\text{max}}$  have previously been reported to increase seasonally with leaf temperature. In one study on six *Eucalyptus* species, measurements were taken at six temperature levels in winter, spring and summer; there was an increase in  $V_{\text{cmax}}$  and  $J_{\text{max}}$  with air temperature in seasons with overlapping temperatures, and  $V_{\text{cmax}25}$  was significantly higher in the winter than in the summer (Lin et al., 2013). Miyazawa and Kikuzawa (2006) obtained similar results in five evergreen broadleaved species. Our measurements also yielded similar results (Figs. 5 and 7).

#### 4.3. Seasonal variability in leaf nutrients

Levels of leaf N and P have been reported to change seasonally (Medlyn et al., 2002a, and Fig. 9). We found a reduction in the N:P ratio in the warm season, consistent with a reduced allocation of N to photosynthetic functions (Way and Sage, 2008). A reduction in total leaf N does not necessarily indicate changes in N allocation; however, a strong coupling between N and photosynthesis has been widely observed, even though Rubisco accounts for only 10-30% of the total leaf N (Evans, 1989). This coupling can be verified in our data, represented by the relationship between  $V_{\text{cmax}}/N_{\text{area}}$  and temperature across the two seasons, which was significant for a greater number of species in comparison to the relationship between  $V_{\text{cmax}}$  and temperature.

A reduction of leaf N in the warm season is unlikely to be caused by general growth dilution during an actively growing part of the year because: a) this is an environment with a year-round growing season; b) PPFD during the periods of the field campaigns was similar; and c) the measured  $V_{\text{cmax}}$  values were shown to be consistent with the coordination hypothesis, implying similar assimilation rates in the two seasons. We did not find significant relationships of photosynthetic traits to foliar N or P despite the study area being extremely limited in supplies of both nutrients (Prober et al., 2012).

#### 4.4 Links between photosynthetic activity, $R_{\text{dark}}$ and $c_i:c_a$

$V_{\text{cmax}}$ ,  $J_{\text{max}}$  and  $R_{\text{dark}}$  were positively correlated with leaf temperature across a wide range (cool season 17 to 27°C; warm season 26 to 37°C), both for the dataset as a whole and within individual species (Figs. 5 and 7). Photosynthetic capacity and respiratory flux are linked via the ATP (adenosine triphosphate) demand of sucrose synthesis and transport, leading to the interdependence of chloroplast and mitochondrial metabolism (Krömer, 1995; Ghashghaie et al., 2003). The parallel temperature acclimation of  $R_{\text{dark}}$  and  $V_{\text{cmax}}$  illustrates this close relationship.

Across all species, there was a strong negative relationship between  $c_i:c_a$  and photosynthetic capacity. A simple interpretation of this (common) finding is that a higher photosynthetic capacity allows a stronger drawdown of  $\text{CO}_2$  between  $c_a$  and  $c_i$ . However, this interpretation assumes that a higher  $V_{\text{cmax}}$  means a higher assimilation rate – which cannot be true if  $V_{\text{cmax}}$  is already optimized to the current light environment. Instead, the coordination hypothesis implies that the lower the  $c_i:c_a$  ratio, the greater the photosynthetic capacity that is *required* in order to achieve a given assimilation rate. Measured  $c_i:c_a$  ratios in this study did increase with temperature as predicted, but the slope of 0.006 (Fig. 6) based on all data was shallower

than the predicted slope of 0.013. This difference may reflect higher vVPD in the warm season, which would be expected to close stomata and therefore act in the opposite way to the effect of temperature alone (Prentice et al., 2014).

#### 4.5 Implications for modelling

Data available to test predictions of the coordination hypothesis regarding the seasonal acclimation of  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and  $R_{\text{dark}}$  are scarce. This study has presented evidence suggesting that such acclimation (at least in evergreen woody plants) is a real phenomenon, and is quantitatively consistent with the coordination hypothesis. Terrestrial models that do not allow seasonal acclimation therefore may incorrectly represent the seasonal time course of carbon exchange at the plant and ecosystem levels.

One dynamic global vegetation model, the Lund-Potsdam-Jena (LPJ) model (Sitch et al., 2003), together with several later models based on LPJ, formally assumes the coordination hypothesis (as well as the coupling between  $R_{\text{dark}}$  and  $V_{\text{cmax}}$ ) and thus implicitly allows photosynthetic parameters and leaf respiration to acclimate to the seasonal variation of climate. The coordination hypothesis is also invoked by the new ‘first-principles’ global primary production model developed by Wang et al. (2014) and further elaborated and tested by Wang et al. (2017). However, there are several steps between these models and the potentially wider application of the coordination hypothesis in physical land surface and Earth System modelling. Time scales are a key issue. The time scale of acclimation of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  is unclear, and could not be resolved empirically without frequent repeat measurements. It is generally accepted, however, that acclimation occurs over multiple days. The LPJ model requires daily or monthly inputs and assumes complete acclimation on monthly time scales. Wang et al. (2014, 2017) implemented their model on monthly timesteps. Modelling diurnal cycles would require a separation of time scales, such that photosynthetic capacities would be near-constant over a diurnal cycle but would vary gradually in response to the seasonal cycle. Thus, whereas Wang et al. (2014, 2017) could make  $V_{\text{cmax}}$  and  $J_{\text{max}}$  ‘disappear’ from equations describing monthly primary production (because on this time scale they were considered to depend only on the environment), multitemporal applications will have to explicitly predict time-varying values of  $V_{\text{cmax}}$  and  $J_{\text{max}}$  depending on antecedent environmental conditions. Field measurements of photosynthetic parameters in different seasons are required to test these predictions (Bloomfield et al., 2018).

#### 25 Data sets

Data can be requested at the Terrestrial Ecosystem Research Network (TERN) data discovery portal <http://portal.tern.org.au> (Prober et al. 2015).

## Author contributions

H.F.T, I.C.P, O.K.A, C.M, and S.M.P planned and designed the research. H.F.T performed all research and data analysis. H.F.T measured all the field data used in the experiment. I.C.P. developed the theoretical predictions. H.F.T wrote the first draft; all authors commented on subsequent versions and assisted with data interpretation.

## 5 Competing interests

The authors declare that they have no conflict of interest.

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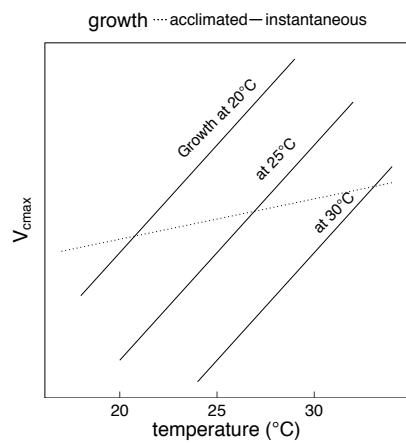


## Tables

**Table 1:** Linear regression slopes ( $K^{-1}$ ) and their 95% confidence intervals for natural log-transformed photosynthetic traits, with species included as a factor. The values are compared to 'kinetic' slopes (as expected in the absence of acclimation) and 'acclimated' slopes, as predicted by the coordination hypothesis for  $V_{\text{cmax}}$ ,  $J_{\text{max}}$  and  $R_{\text{dark}}$  and the least-cost hypothesis for  $c_i:c_a$ .

	$V_{\text{cmax}}$	$J_{\text{max}}$	$R_{\text{dark}}$	$c_i:c_a$
<i>Fitted</i>	0.0328	0.0251	0.0514	0.0060
<i>s.e.</i>	(±0.0158)	(±0.0108)	(±0.0164)	(±0.0033)
<i>Kinetic</i>	0.0885	0.0628	0.0675	n/a
<i>Acclimated</i>	0.0493	0.0236	0.0494	0.0131

## Figures



5 Figure 1: Schematic showing the difference between instantaneous and acclimated  $V_{\text{cmx}}$  as predicted by the coordination hypothesis. The three solid lines indicate the instantaneous response of  $V_{\text{cmx}}$  to temperature for plants grown at 20°C, 25°C and  
10 30°C under identical light conditions. The dashed line indicates the predicted response of field-measured (acclimated)  $V_{\text{cmx}}$  to the growth temperature. The instantaneous responses are due to enzyme kinetics, whereas the acclimated response is due to the slightly higher  $V_{\text{cmx}}$  required to compensate for increased photorespiration at higher temperatures. Because the acclimated response is shallower than the instantaneous response,  $V_{\text{cmx}}$  at a common temperature (e.g. 25°C) declines with growth temperature.

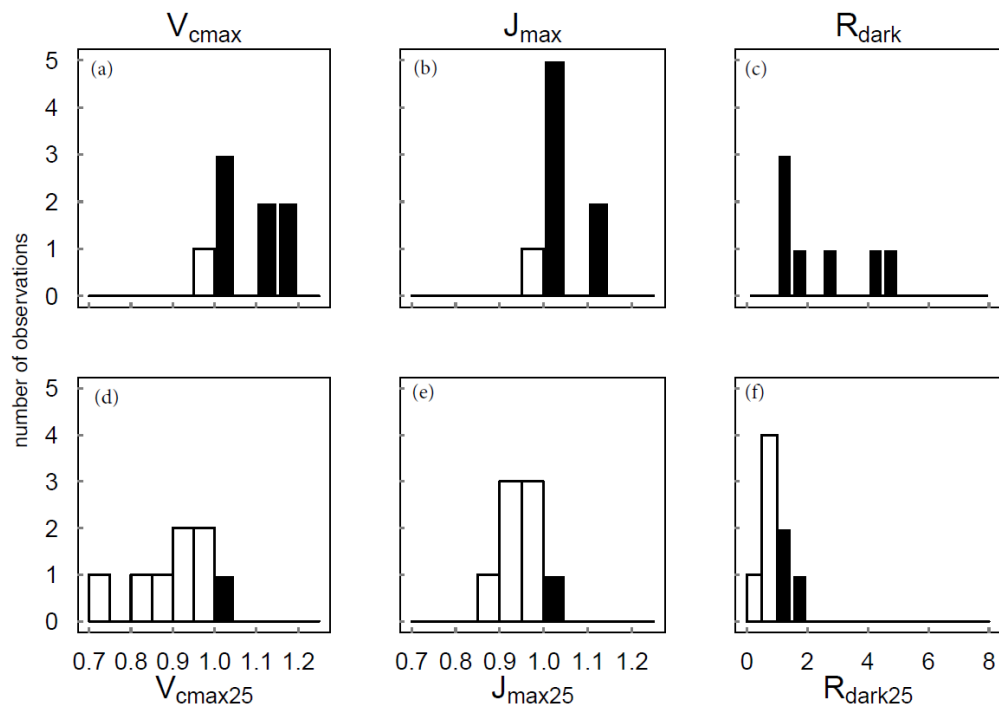


Figure 2: Distributions across species of the warm:cool season ratios of  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  measured at ambient temperature (top panels) and standardized to 25°C (bottom panels). Ratios > 1 are shown as black bars, ratios < 1 as white bars ( $n = 8$ ).

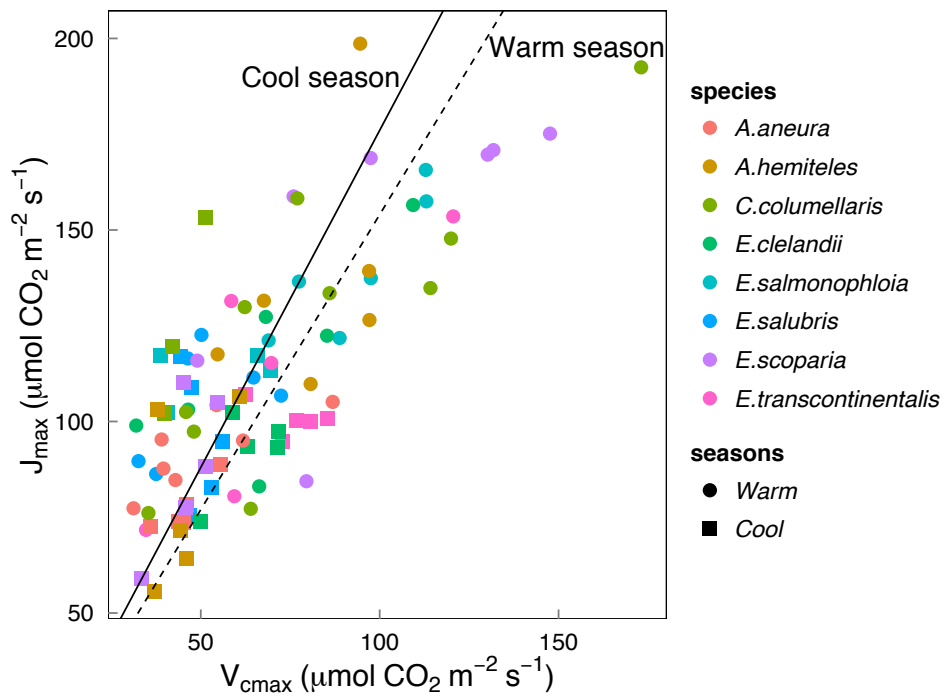


Figure 3: Linear regressions forced through the origin between  $J_{\max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and  $V_{\text{cmax}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) for individual trees of eight species in the warm season (circles, dashed line, slope = 1.58) and the cool season (squares, solid line, slope = 1.79). Both regressions are significant ( $p < 0.05$ ). Each point represents one *A-c*<sub>i</sub> curve ( $n = 86$ ).

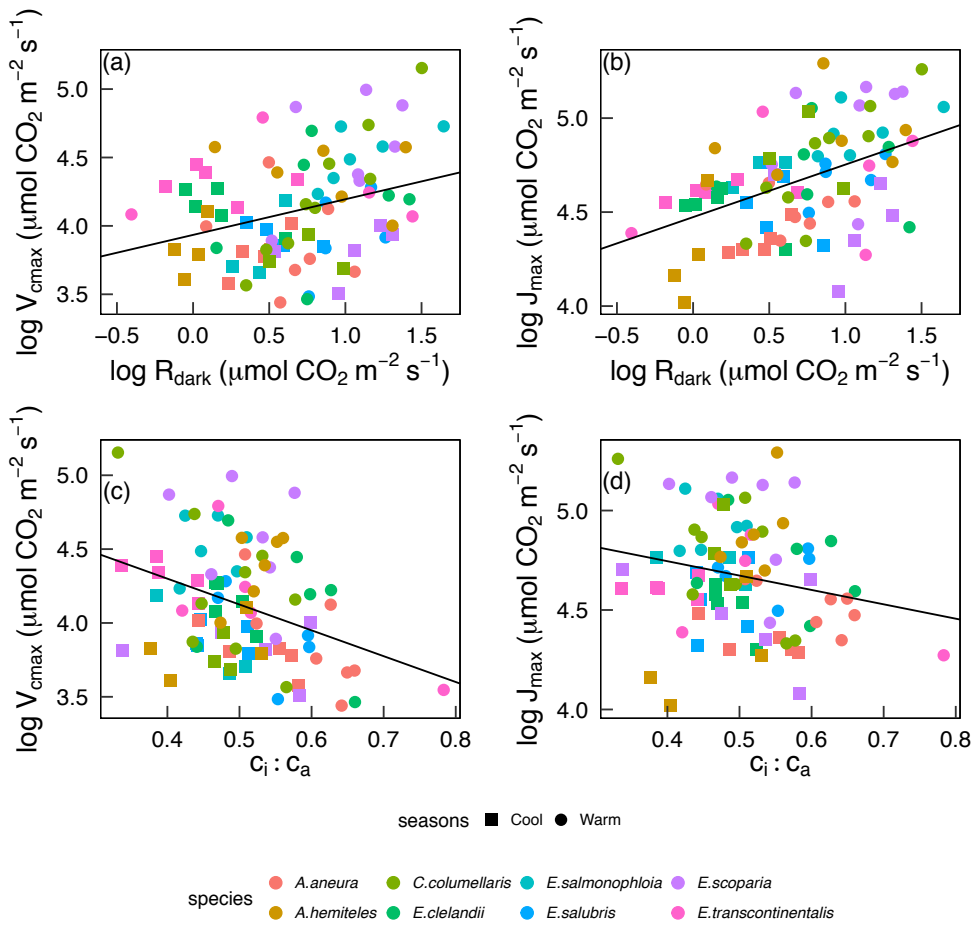
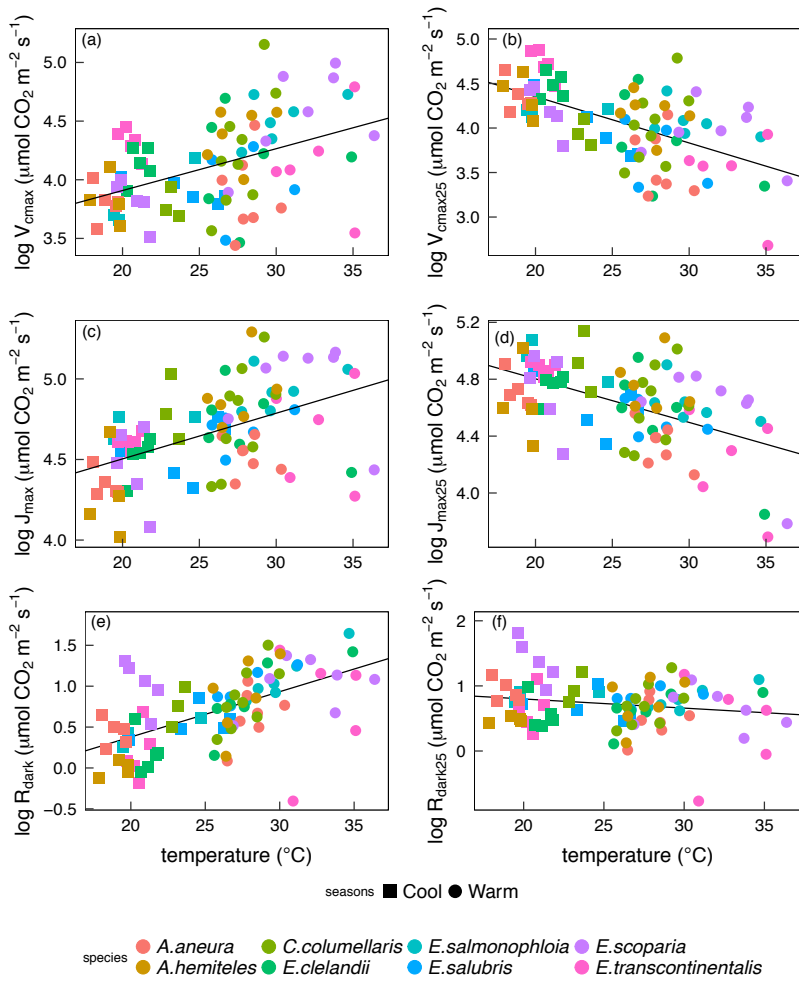


Figure 4: Linear regressions of individual trees by season ( $n=16$ ) and species ( $n=8$ ) for photosynthetic capacity,  $V_{cmax}$  and  $J_{max}$  with  $R_{dark}$  ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ ) and the  $C_i:C_a$  ratio at ambient  $\text{CO}_2 \approx 400 \mu\text{mol mol}^{-1}$  ( $p < 0.05$ ).  $V_{cmax}$ ,  $J_{max}$  and  $R_{dark}$  were  $\log_{10}$  transformed;  $C_i:C_a$  was not. Each point represents one  $A-c_i$  curve ( $n=86$ ).

5



**Figure 5: Bivariate linear regressions ( $p < 0.05$ ) of natural log transformed  $V_{cmax}$ ,  $V_{cmax25}$ ,  $J_{max}$ ,  $J_{max25}$ ,  $R_{dark}$  and  $R_{dark25}$  ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) versus leaf temperature ( $T_{leaf}$ , °C). Each point represents one  $A-c_i$  curve ( $n = 86$ ).**

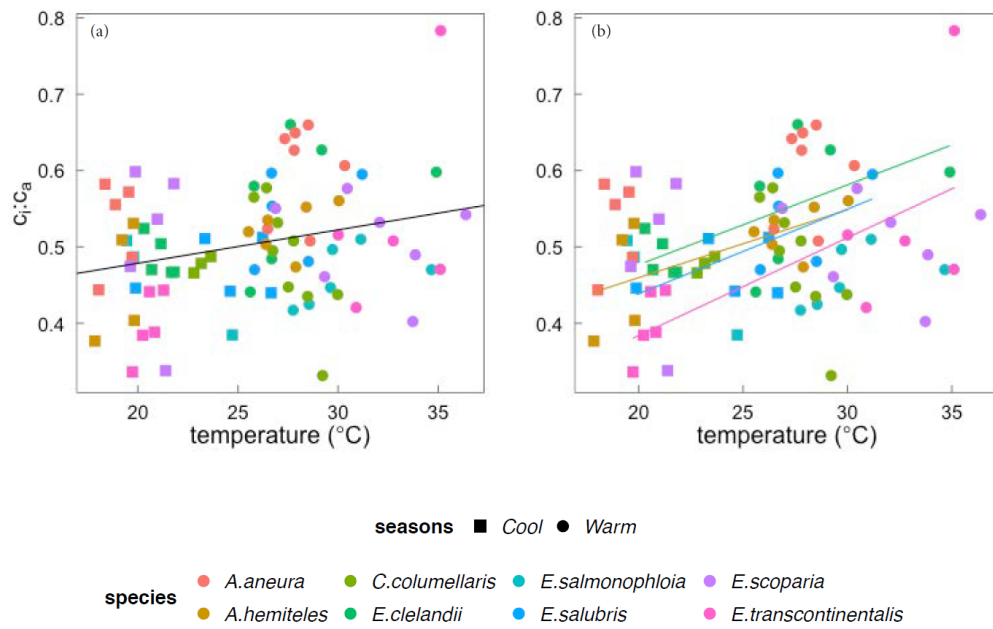
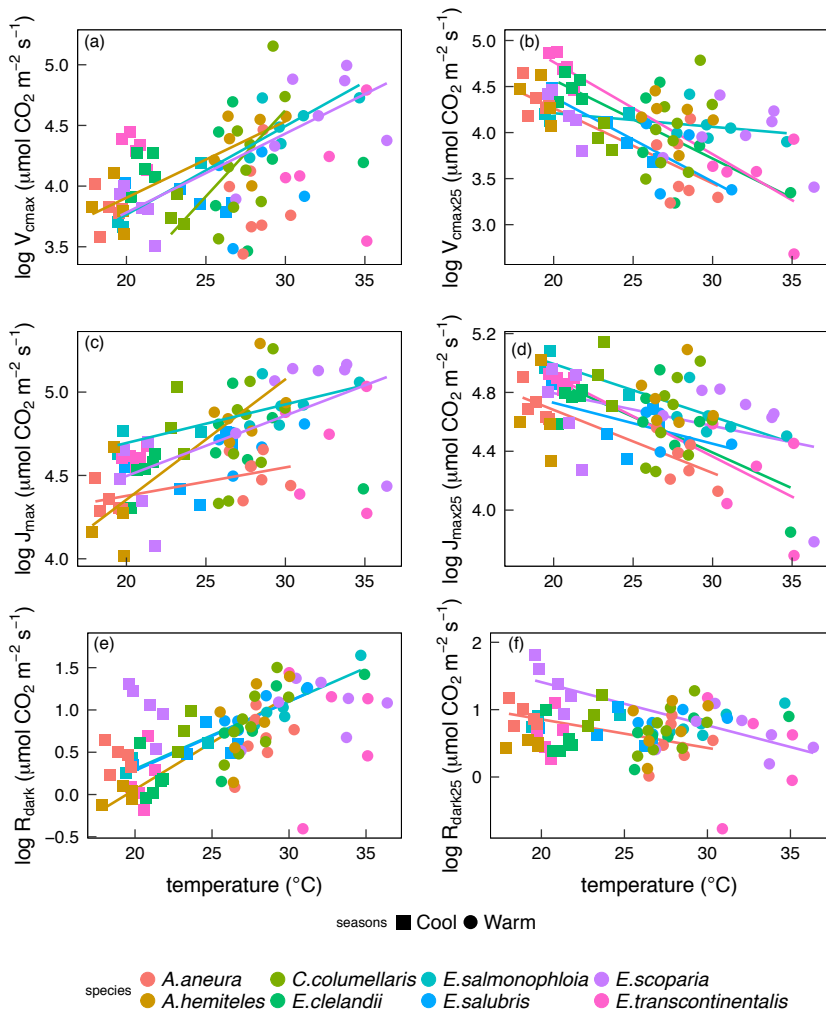


Figure 6: Bivariate linear regressions of the  $c_i:c_a$  ratio (at ambient  $\text{CO}_2 \approx 400 \mu\text{mol mol}^{-1}$ ) versus temperature ( $T_{\text{leaf}}$ ,  $^{\circ}\text{C}$ ) for individual trees considering all data (a) and within species (b). Only significant regressions ( $p < 0.05$ ) are shown. Each point represents one  $A-c_i$  curve ( $n=86$ ).



**Figure 7: Bivariate linear regressions of natural log-transformed  $V_{\text{cmax}}$ ,  $V_{\text{cmax}25}$ ,  $J_{\text{max}}$ ,  $J_{\text{max}25}$ ,  $R_{\text{dark}}$  and  $R_{\text{dark}25}$  ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) versus leaf temperature ( $T_{\text{leaf}}$ , °C) within species ( $p < 0.05$ ). Only significant regressions ( $p < 0.05$ ) are shown. Each point represents one  $A-c_i$  curve ( $n = 86$ ).**



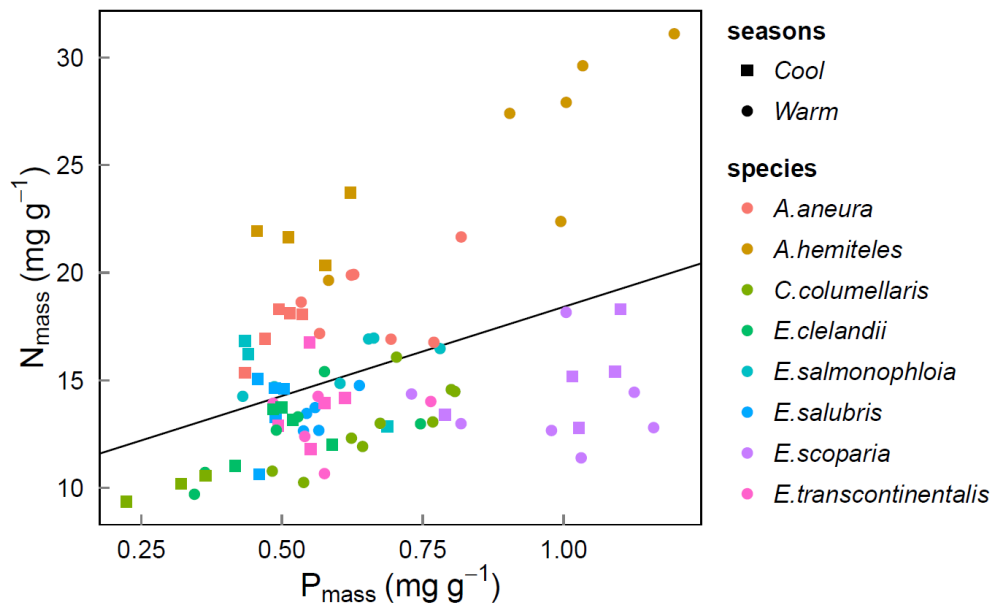


Figure 8: Bivariate linear regression of  $N_{mass}$  ( $\text{mg g}^{-1}$ ) versus  $P_{mass}$  ( $\text{mg g}^{-1}$ ) for all data (black line, slope = 0.33,  $R^2 = 0.17$ ). Each point represents one leaf ( $n = 86$ ).

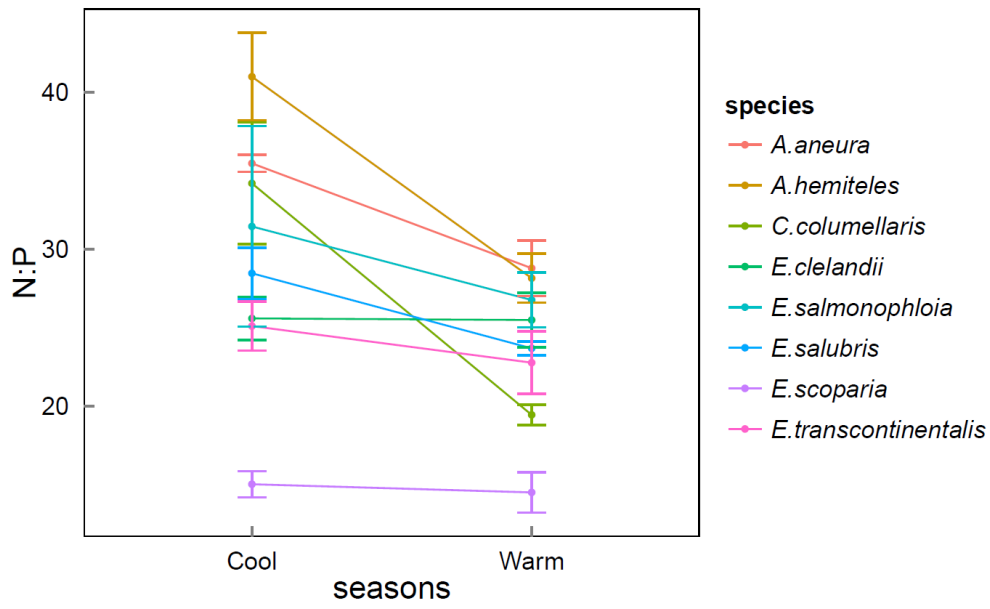


Figure 9: Changes in the average foliar N:P ratio for each species between the cool and the warm seasons. Standard errors shown.

We thank the reviewers for comments and suggestions that have helped to improve our manuscript. Our response is organized by addressing each comment one by one.

### **Anonymous Referee #1**

**AR1.1.** I find that while the results from the photosynthetic measurements are interesting and C1 well analysed and discussed, the theoretical analysis and the link between measurements and theory needs more discussion, in particular in relation to the many linear assumptions made, all of which are hidden in the appendix.

We have moved the equations to the main text and provided further discussion there. The text now includes the specific rationale for the linear assumptions that we made.

**AR1.2.** The conclusions relating to acclimation and coordination are based on the slopes of regression lines of photosynthetic variables to temperature but there is insufficient detail in the paper relating to the results of the statistical analysis. Figures 3-5 are presented without any goodness of fit metrics or p-values for the individual lines.

We believe this comment refers to Figures 6 and 7. But here, only significant individual lines ( $p < 0.05$ ) are shown, as is described in the Figure captions and in the Results section (3.2).

**AR1.3.** In addition, the authors assume a linear relationship between the log10 values of each variable and temperature, an assumption which is detailed in appendix A but not sufficiently discussed in the main text.

Linear regressions are used because the theoretical equations relating log-transformed traits to temperature are linear. This should now be clear from the revised text, in which the equations are presented up-front. We have also made a specific statement about this matter in Section 2.5 in order to make the point absolutely clear.

**AR1.4.** The coordination hypothesis states that the Rubisco and electron transport limited rates are co-limiting under average conditions, which is generally taken to mean that there is a change in the  $J_{max25}$  to  $V_{cmax25}$  ratio and implicitly a change in nitrogen allocation inside the leaf. The authors make a linear approximation to solve for this co-limitation (eq. A3). This approximation removes the parameter  $J_{max25}$  from the calculation and its slope with temperature is calculated assuming proportionality to the slope of  $V_{cmax25}$  and a ratio of the biochemical temperature response. While these approximations can be justified, I believe that a further discussion is needed as the resulting equations are difficult to match with the coordination hypothesis as this is generally understood.

The central quantitative evidence is the acclimation in  $V_{cmax}$ , which is consistent with theoretical predictions. Nonetheless, our results are also consistent with a simple additional hypothesis about the acclimation of  $J_{max}$ , namely the maintenance of a

constant ratio between  $V_{\text{cmax}}$  and  $J_{\text{max}}$  at a prescribed temperature (e.g. 25°C). Our revision has clarified these matters.

However, while the reviewer might possibly be right to suggest that “it is generally taken” that the co-ordination hypothesis is about the **partitioning** of leaf N to  $J_{\text{max}}$  versus  $V_{\text{cmax}}$ , our understanding is that it only states that **the two limiting rates of photosynthesis tend to be co-limiting under average conditions**. Limitation by  $J_{\text{max}}$  is not normally reached under natural conditions; at low light photosynthesis is limited by  $J$  (not  $J_{\text{max}}$ ), and at high light it becomes limited by  $V_{\text{cmax}}$ . **So the first-order prediction of the co-ordination hypothesis is simply that  $V_{\text{cmax}}$  should acclimate to the average conditions**, and that is our first-order result.

The co-ordination hypothesis thus makes a stronger prediction about leaf N than merely its partitioning to different functions. It actually implies that there is an **optimal value** of leaf N. This has been proposed in a number of papers that we cite, starting back in the 1990s (e.g. Dewar, 1996, predicts an optimal canopy nitrogen using the maximum NPP hypothesis where gross photosynthesis is subtracted by maintenance and growth respiration) and elaborated and evaluated more recently e.g. by Maire *et al.* (2012) and Dong *et al.* (2017).

**AR1.5.** I would also suggest including all the equations in the main body of the text since they are necessary to the central message of the paper

Done.

**AR1.6.** The authors report the slope of the log10 of each measured parameter with temperature and compare this to the theoretical equivalent slope (Table 1) to reach the conclusion that the coordination hypothesis is valid. The more usual approach would be to calculate the theoretically predicted values of the photosynthetic parameters and plot these together with the measured values. The authors’ approach is scientifically valid but given the multiple approximations and log values I find it hard to follow.

We agree that plots are easier to follow than tables; however, we do present partial residual plots – please bear in mind that to maximize statistical power we have analysed the data together in a generalized linear model, using species as factors (as explained in the Methods – Statistical Analysis section, and the captions). We tried various ways to present the data and we found that the approach we have adopted here was the most accessible. We do not agree that a simple comparison of predicted and measured values would be preferable, because it would not convey anything about the mechanistic basis for data-model agreement. Our presentation of relationships to temperature carries the stronger message that these relationships exist in the data.

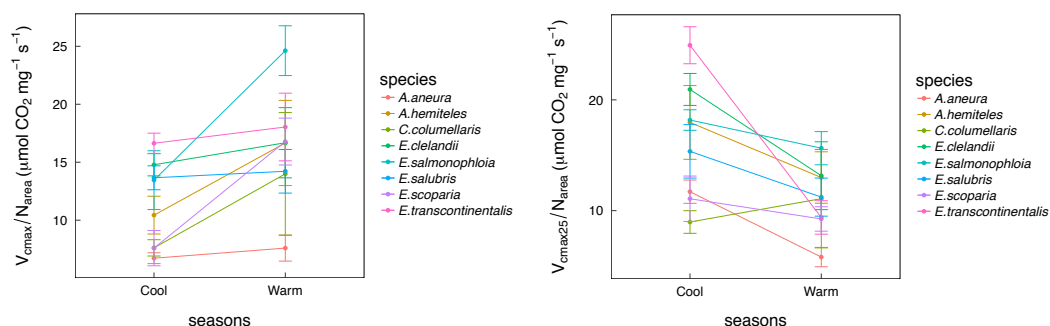
**AR1.6.** Also, the fitted slopes for all parameters are calculated as log10(parameter) vs. temperature, while the theoretical slopes are ln(parameter) vs. temperature. I would suggest that the authors check their calculations and verify that these slopes are equivalent.

This was a slip in the original version. All slopes (observational and theoretical) were calculated using natural logarithms. We have changed the text and Figures to make sure this is clear now.

**AR1.7.** Changes in  $V_{cmax}$  values alone do not verify the coordination hypothesis - these can be caused either by acclimation or by changes in total leaf nitrogen. According to Fig. 8 there are large differences in the leaf N for some species, which can be caused by a number of factors apart from temperature acclimation, especially leaf ageing. I would be interested to see how the ratio of  $V_{cmax}$  (and/or  $J_{max}$ ) to leaf N changes seasonally, which would give a better indication of photosynthetic coordination.

We do not agree that change in total leaf N could meaningfully be a “cause” of changing  $V_{cmax}$ . The co-ordination hypothesis implies (as pointed out by many authors, see remarks above) that there is an **optimal leaf N** for any given set of average environmental conditions. Thus, acclimation involves changes in  $V_{cmax}$  and potentially changes in leaf N as well.

Nonetheless, in response to this comment, we have carried out additional analyses on how the ratio of  $V_{cmax}$  (and  $V_{cmax25}$ ) to  $N_{area}$  varies with growth temperature. The results are generally very similar to the analysis of  $V_{cmax}$  (and  $V_{cmax25}$ ), but they are significant for more species (7/8: all except the gymnosperm *Callitris columellaris*, for both  $V_{cmax}$  and  $V_{cmax25}$ ). We have clarified the point, and referred to this additional analysis, in our revised text. See also the graphs below:



**AR1.8.** While acclimation of respiration is a well documented and important process it is unclear how this links to the coordination hypothesis. Here the authors hypothesised that dark respiration scales linearly with  $V_{cmax}$  and will therefore follow the coordination hypothesis as well, but this is not necessarily the case in either models or reality and a better justification of why the variation in dark respiration should be linked with photosynthetic co-limitation is needed.

The reviewer is correct to indicate that to predict the acclimation of  $R_{dark}$  to temperature from the co-ordination hypothesis requires an additional hypothesis. We have tried out the simplest, i.e. that  $R_{dark}$  remains proportional to  $V_{cmax}$ . This logic, which is also used in many models, is now explicitly spelled out.

**AR1.9.** The authors should decide whether we are talking about ‘coordination’ or ‘co-ordination’.

The spelling ‘co-ordination’ has been replaced by ‘coordination’.

## **Anonymous Referee #2**

**AR2.1.** My main comment is that the discussion is very thin. It could use more substance and less reiterating the results. What do you make of the considerable spread in the data? Why do many species in Figure 6 not show the expected response, even if the pooled data does? There’s a lot more here to discuss than is currently covered.

We have expanded the discussion explaining light limitation of  $J_{max}$  as well as relationships of photosynthetic traits to nitrogen and temperature across the two seasons. We have also expanded the discussion of implications for modelling. However, we do not have any useful information on the causes of differences among species. This is normal for trait analyses! Our goal has been to uncover general, first-order relationships. At this stage it is not surprising that not every species conforms to a universal pattern.

**AR2.2.** There are a number of studies that have measured  $V_{cmax}$  and  $J_{max}$  at multiple times across a season in the literature (Baldocchi has a few, for example). These should be acknowledged in the intro. Similarly, there should be a citation to Way and Yamori 2013 who found no change in  $V_{cmax25}$  in a meta-analysis of plants grown at different temperatures.

We have now included the suggested citations. A study from 2000 by Wilson and collaborators, including Baldocchi, was cited as example of data on the responses of photosynthetic traits on ecologically relevant time scales. We have also included a mention of Way and Yamori’s differing findings in the Discussion.

**AR2.3.** Why was  $R_{dark}$  measured after only 5 mins in the dark? This is usually measured after at least 20 and often 30 minutes of darkness to get a true estimate of dark respiration.

This was a time-saving compromise to allow four or five replicate curves per machine per day, based on our experience that stable results are commonly obtained after 5 minutes. Moreover, this quick estimate should still be superior to the common practice of deriving  $R_{dark}$  as one of the parameters in a curve-fitting routine. We have added a comment to this effect.

**AR2.4.** What VPD were the measurements made at? If the summer VPD is higher,  $g_s$  will be reduced, which will lower the  $C_i/C_a$  ratio, but this isn’t necessarily a temperature effect per se.

There is indeed a systematic difference between VPD in the two seasons. The average VPD value during the warm season was 1.13 kPa, and during the cool season 0.45 kPa. The average *leaf-to-air* VPD (i.e. corrected to leaf temperature) during the

warm season was 2.5 kPa, and during the cool season 1.44 kPa. However, there was very little difference in stomatal conductance at light saturation: (0.064 – 0.082 – 0.101) (lower quartile – median – upper quartile) mol m<sup>-2</sup> s<sup>-1</sup> for the warm season, and (0.057 – 0.078 – 0.085) mol m<sup>-2</sup> s<sup>-1</sup> for the cool season. We have added a note on this.

**AR2.5.** Figure 2 - why were the fits forced through the origin and how does this affect the slopes? Is it a minimal effect?

Both slopes are significant if not forced through origin. However, an intercept for this relationship is (a) extrapolated, and (b) makes no biological sense. We have added a note on this.

**AR2.6.** Lastly, while I appreciate the use of the log-transformed data to get linear slopes, I'd like to see the "real" data, at least in the SI. This makes it much easier to see the values measured and compare the data with the majority of other studies that report V<sub>cmax</sub> and J<sub>max</sub> values against leaf temperature.

The real values are in fact presented in the Results section, and in Figure 2. Raw data are also available on the TERN portal as mentioned in the text.

Technical comments

**AR2.6.** Page 2, Line 13 - please clarify what "these" refers to - V<sub>cmax</sub> and J<sub>max</sub>, yes?

Yes. We have clarified this now.

Page 9, Line 7 - the relationship between C<sub>i</sub>/C<sub>a</sub> and photosynthetic capacity could also be because higher photosynthetic capacity (at a constant g<sub>s</sub>) reduces C<sub>i</sub>. Cause and effect can't be determined.

We have added a note on this alternative explanation in Discussion (section 4.4). It assumes that higher V<sub>cmax</sub> means more photosynthesis.... but if the coordination hypothesis is correct, then this assumption is incorrect.

If all the gas exchange is determined with a Licor IRGA, how are the parameters being reported in units of electrons and O<sub>2</sub>? J<sub>max</sub> and R<sub>dark</sub> should be in units of CO<sub>2</sub> per area per time.

We have amended the units as suggested.

### **Anonymous Referee #3**

**AR3.1.** What is the role of phenology / specifically leaf age here, there is a need to discuss this either in the introduction and or discussion, i.e. there might be confounding phenological and thermal acclimation effects in the presented results. What is the life time of a leaf in this semi-arid evergreen woodland? -Related to the above, the manuscript provides an explanation of changes in N:P ratios from cold to

warm season in Fig 8 however it does not explain how these changes happened, how did leaf N and P changed and how this might be related to leaf age? It would be good to add a plot showing individual values of leaf N and Leaf P in the cold and warm season.

Three factors militate against any important role for phenology and leaf ageing in our results. First, the generally long (5-10 years) lifetime of leaves in this ecosystem, meaning that ageing proceeds slowly; second, there is **continuing turnover of leaves** through the year in this ecosystem; and third, we sampled **the youngest fully expanded leaves** in both seasons.

**AR3.2.** Equation 1 is used to estimate  $V_{cmax}$  and  $J_{max}$  at 25C. During the warm period (unclear time of day A-Ci curves where taken)  $V_{max}$  at T could be either in the optimum or beyond the optimum temperatures, thus it is possible that the peaked temperature response might be more appropriate. If this was the case, how is this likely to affect the results? Also, how does the choice of  $H_a$  (Medlyn et al 2002) value affects the results. According to Hikoska et al (2006) there is a relationship between activation energy of  $V_{cmax}$  and growth temperature. Similar comments apply to the use of equation A2 to determine the slope of  $V_{cmax}$  and temperature presented in Table 1 under the kinetic approach. Is the slope sensitive to the choice of  $H_a$  but most important are the slope values robust when estimated with the peaked temperature response for  $V_{cmax}$  and  $J_{max}$ .

In theory  $T_{opt}$  can affect the calculation of  $V_{cmax}$  and  $J_{max}$ . However, Medlyn et al. (2002), Kattge & Knorr (2007), and others have found very good correlations between  $T_{opt}$  and mean daily temperature. We measured A-Ci curves between morning and early afternoon, avoiding the hottest part of the day. Therefore, it is unlikely that any of our measurements were carried out above the optimum temperature. We have added a comment to this effect.

Regarding the activation energy of  $V_{cmax}$ , there have been reports of a sensitivity to growth temperature but any such effect appears to be small and many studies have failed to find any such effect (e.g. Kattge & Knorr, 2007). No information was available on  $H_a$  for the species sampled and so (in common with much of the ecophysiological literature) we adopted a generic *in vivo* value. We recognize that the activation energy of  $J_{max}$  is much more variable with growth conditions.

**AR3.3.** Leaf dark respiration measurements were taken after only 5 minutes of leaves being in the dark. Protocol for  $R_d$  estimates is at least 30 min in the dark (Atkin et al 2000; Atkin et al 1998) as it takes about 15-20 minutes for post-illumination respiration to stabilize with time increasing with decreasing temperature. How does this affect your measurements of  $R_{dark}$  and acclimation results?

This was a time-saving compromise (see our response under AR2.3 above).



**AR3.4.** On the implications for modelling section it would be very relevant to apply the Kattge & Knorr (2007) formulations and compare to your data set and predictions by the optimization approach used in this study.

See our response to AR3.2 above.

**AR3.5.** Is the data from this study consistent with the  $V_{cmax25}$  prediction derived by Scafaro et al (2017)

Yes. This was mentioned in the original text, but the reference was missing in the bibliography. We have rectified this omission.

**AR3.6.** Either in the introduction or in the methodology, it would be good to include a graphic explaining the change in temperature responses to illustrate what acclimation is, i.e. temperature response shifts forward and therefore values at 25 C decline, you could illustrate also where in the curve are the leaf temperature values are during the cold and warm season.

We have provided the suggested graphic, as the new Fig. 1.

**AR3.7.** It would be useful to include a figure of the mean diurnal cycle of air temperature during the warm and cold seasons but also provide an idea of when the A-CI curves were taken and under which RH, VPD conditions. If RH & VPD conditions differ, what are the implications.

The Methods section already provides information on the diurnal cycle of temperature in both seasons, and the timing of our measurements. Regarding the potential effects of VPD, please see our response under AR2.4 above.

**AR3.8.** P10, L 25 Can you clarify in the text why the acclimated slope of  $J_{max}$  to leaf temperature was estimated as the acclimated slope of  $V_{cmax}$  minus the difference of the kinetic slopes of  $V_{cmax}$  and  $J_{max}$  (this might also be affected by peaked temperature response)

We have now explained exactly what we are doing here in the revised text. It is a simplification, as described in our response to AR1.4 above, but we hope now it is clear.

**AR3.9.** P3 L2 –can elaborate here and explain homeostasis

We have changed the wording here, to be more explicit, and avoided the term homeostasis.

**AR3.10.** P6 L22 Is this  $T_{leaf}$  measured by the Licor or an independent measurement? If yes would be good to mention it in the methods section

$T_{leaf}$  is the leaf temperature as measured by the LiCor. We have noted this in the text now.

**AR3.11.** P7 L26-29 These values were not really shown as it was all logged transformed, would be nice to show the data.

The values are presented in the Results section and in Figure 2. Raw data are also available on the TERN portal, as mentioned in the text. Since the manuscript already includes nine Figures and one Table, we prefer to keep the reader's attention focused on the main results.

**AR3.12.** The sentences comparing values to desert plants and mesic perennial species could be more specific and include typical values for those vegetation types otherwise is all very generic and less informative.

We have included some example values.

**AR3.13.** P8 L6 but 'lower allocation of N to Rubisco' has not been demonstrated here.

We agree, but this is presented in the text here as a prediction, not as a fact!

**AR3.14.** P8 L9, need to mention the role of leaf age /phenology, maybe here good to show N values change and use this to support some of the sentences on this paragraph.

Please see our response to AR 3.1.

### **Short Comment #1**

**SC1.1.** Based on their data or via model simulations, suggest how the ecosystem models can be improved. That is, if you were to use an ecosystem model, how would the parameters that you measured change with time in the model. In my view, coordination hypothesis has already been implemented in some ecosystem models.

We have added some wording to address this point in the Discussion.

**SC1.2.** You have the seasonal data and you just connect two points in Fig. 8. First in my view, this does not seem right. It would be nice to show better the temporal variation of the parameters for these evergreen species. My main concern here is to specify how much is the variation in the parameters of these evergreen species due to the different seasons e.g. 10%, 20%, etc.

We were not monitoring the species through a whole year, and so it is not possible to provide what is asked for here. However, we have provided some indication of the relative magnitude of seasonal changes in parameters.

**Associate Editor**

**AE.1.1.** Answer 1.4: I find this answer only partially convincing because if I follow your line of argumentation, in light saturation, photosynthesis is not limited by  $V_{cmax}$ , but by  $V_c$ , as there is still a  $C_i$  dependence to be respected.

Of course the  $C_i$  dependence of photosynthesis is important, but it doesn't alter our argument. The coordination hypothesis indicates that under typical daytime conditions, the Rubisco-limited and electron transport-limited rates of photosynthesis should be approximately equal, **given a set of conditions that include  $C_i$**  (and its influence on both rates). And because light is 'external' to the plant, the first-order prediction of the coordination hypothesis is that  $V_{cmax}$  must adjust to the available light. We hope we have now made this clear in the text, while avoiding too much distracting complexity. See our response to AR 1.4 above.

**AE.1.2.** Answer to comment 2.1/2.2: This answer is too superficial for the open discussion. An outline of your responses to these comments would have been appropriate. Please be sure to provide more detail in your response letter

Please see our extended responses to AR 2.1 and 2.2 above.

## **Recommendations to the editor: Report 2**

**R2.A:** I would like to thank the authors for their detailed response to my comments. The only issue that I do not think has been addressed sufficiently is the definition and implications of the coordination hypothesis which lies at the basis of this paper. While the authors correctly say that the coordination hypothesis simply states that the two limiting rates of photosynthesis,  $A_v$  and  $A_j$  should be equal, there are two opposing interpretations of this statement. The first one, which the authors also subscribe to, is that the  $J_{max}/V_{cmax}$  ratio remains constant and the leaf N changes (Chen et al. 1993; Maire et al. 2012). The alternative is of course that the leaf N remains constant and the  $J_{max}/V_{cmax}$  ratio varies (Ali et al. 2015; Medlyn 1996; Quebbeman and Ramirez 2016). These two contrasting approaches should be mentioned in the introduction and the methods and which of these the authors use in their model analysis.

We have made the inclusion suggested.

As a side note, the (Dewar 1996) study which the authors mention in their reply, does indeed predict an optimal canopy nitrogen but does so using the maximum NPP hypothesis, which is distinct and must not be confused with the coordination hypothesis.

We included a note as suggested on our response to AR.4