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

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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.

1 Introduction

Net photosynthetic CO_2 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 serial 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 (Wullschleger, 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 (Scafaro et al., 2017). Scafaro et al. (2017) also

demonstrated that the acclimation of V_{cmax} involves changes in Rubisco amount, and the relative allocation of leaf N to Rubisco. If it is further assumed that J_{max} covaries closely with V_{cmax}, as is commonly observed, then similar predictions apply to the acclimation of J_{max}. Wang et al. (2017) have provided a fuller treatment of J_{max} acclimation, based on a cost-minimizing principle that also correctly predicts the dependence of the ratio J_{max}/V_{cmax} on growth temperature (Kattge and Knorr, 2007; Wang et al., 2017).

R_{dark} (leaf respiratory 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_{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_{dark} at 25°C increasing upon cold acclimation and declining upon acclimation to warmer temperature (Reich et al., 2016). Growth temperature-dependent changes in R_{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_{dark} (Atkin et al., 2015) showed that geographic variation in R_{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_{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_{dark} are consistent with the acclimation of R_{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_{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
5   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
10   obtained from the flux tower (TERN Ozflux, 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

15   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
20   close to the air ambient temperature. The 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 μmol 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 μmol m^{-2} s^{-1}. After measuring the A-c_i curves over about 35 minutes, light was set to zero for five minutes before measuring R_{dark}. This was a time-saving compromise to allow four or five replicate curves per machine per day, based
25   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. 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.

30   In theory optimal growth temperature (T_{opt}) could affect the calculation of V_{cmax} and J_{max}. However, Medlyn et al. (2002b) and Kattge and Knorr (2007) have found very good correlations between T_{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 $CO_2 \approx 400$ µmol mol^{-1}.

5 **2.3 Photosynthetic parameters and their temperature responses**

Apparent values of V_{cmax} and J_{max} were fitted using the Farquhar et al. (1980) model. Values were standardized to 25°C (V_{cmax25} 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_{cmax} and J_{max}:

10 $$param\,(T) \; = \; param\,(T_{ref})\;\exp\left[(\Delta H_a/R)\,(1/T_{ref} - 1/T)\right] \tag{1}$$

where ΔH_a is the activation energy (J mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), and T_{ref} is a reference temperature; T is the leaf temperature (K). To derive R_{dark25} 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 $$R_{dark25} = \; R_{dark}\,(3.09\,-\,0.043\left[\frac{T_{Cref}+T_C}{2}\right])^{\left[\frac{T_{Cref}-T_C}{10}\right]} \tag{2}$$

where 3.09 and 0.04 are empirical constants, $T_{Cref} = 25$°C and T_C is leaf temperature (°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 (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; g m^{-2}) was calculated from leaf area and dry mass. N_{mass} and P_{mass} were obtained by Kjeldahl acid digestion of the same leaves (Allen et al., 1974). The leaf
25 material was digested using sulphuric acid 98% and hydrogen peroxide 30%. Digested material was analyzed for N and P using a flow injection analyser system (LaChat QuikChem 8500 Series 2, Lachat Instruments, Milwaukee, WI, USA). Area-based N and P values (N_{area}, P_{area}, mg m^{-2}) were calculated as the products of LMA and N_{mass} or P_{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_{cmax}, V_{cmax25}, J_{max}, J_{max25}, R_{dark}, R_{dark25}, N_{area} and P_{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_{cmax} (Fig. 1), J_{max} and R_{dark} and the least-cost hypothesis for $c_i{:}c_a$. 'Kinetic' temperature responses were predicted from equation (1), which can also be written:

$$\ln param\,(T) \;-\; \ln param\,(T_{ref}) \;=\; (\Delta H_a/R)\,(1/T_{ref} - 1/T) \tag{3}$$

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

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

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

The 'acclimated' response of V_{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:

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

where φ_0 is the intrinsic quantum efficiency of photosynthesis, I_{abs} is the absorbed PPFD, K is the effective Michaelis-Menten coefficient for carbon fixation and Γ^* is the photorespiratory compensation point. The theoretical (acclimated) sensitivity of V_{cmax} to temperature was then calculated from the derivative of equation (5):

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

We evaluated Eq. (6) at $T = 298$ K and $c_i = 200$ µmol mol^{-1} (approximately the median of our observed values of c_i), using the temperature dependencies of K and Γ^* 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_{max} as being the acclimated slope of V_{cmax}, minus the difference between the kinetic slopes of V_{cmax} and J_{max}; this is equivalent to assuming that the ratio of J_{max} to V_{cmax} at 25°C is constant. We further assumed that R_{dark} (on acclimation) should be an invariant fraction of V_{cmax}, implying the same acclimated temperature response for R_{dark} as for V_{cmax}.

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

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

where b is the (assumed constant) ratio of R_{dark} to V_{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, η). Holding vapour pressure deficit (D) constant, we obtained an expression for $\partial \chi^*/\partial T$:

$$\partial \chi^*/\partial T = (\chi^*/2) \, (\partial \ln K/\partial T - \partial a/\partial T) \tag{8}$$

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

$$\partial \chi_o/\partial T = (\chi_o/2) \, (1 - \chi_o) \, (\partial \ln K/\partial T - \partial a/\partial T) \tag{9}$$

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 η.

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 μmol m^{-2} s^{-1}, J_{max} from 77.4 to 160 μmol m^{-2} s^{-1}, R_{dark} from 1.16 to 3.14 μmol m^{-2} s^{-1} and c_i:c_a from 0.39 to 0.60 (at ambient $CO_2 \approx$ 400 μmol 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 = ±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_{cmax} and J_{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_{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_{cmax} and J_{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^{-2} s^{-1} for the warm season, and 0.057 – 0.078 – 0.085 mol m^{-2} s^{-1} for the cool season.

     Incoming shortwave radiation at the surface was used as a proxy for PPFD. Daily values ranged from 90 to 256 W
10 m^{-2} and averaged 193 W m^{-2}. 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_{area} or P_{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_{cmax} (and V_{cmax25}) to N_{area} varied with ambient temperature. The results were generally very similar to the analysis of V_{cmax} (and V_{cmax25}) 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 columelaris*) for both V_{cmax} and V_{cmax25} (not shown).

3.4 Quantitative temperature responses

For V_{cmax} and J_{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_{cmax} can be predicted from the temperature dependencies of K and the photorespiratory compensation point (Γ^*). The acclimated slope for V_{cmax} falls just marginally above the 95% confidence interval for the fitted slope. The acclimated slope of J_{max} falls well within the 95% confidence interval for the fitted slope. For R_{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

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 ± 54 μmol m^{-2} s^{-1}), but higher than typical values for mesic perennial species (53 ± 15 μmol m^{-2} s^{-1}) (Wullschleger, 1993). The values were also high compared with trees from savannas with twice the annual precipitation (range $20 - 90$ μmol m^{-2} 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˚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_{cmax} and J_{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_{cmax} and J_{max} with air temperature in seasons with overlapping temperatures, and V_{cmax25} 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_{cmax}/N_{area} and temperature across the two seasons, which was significant for a greater number of species in comparison to the relationship between V_{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_{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_{dark} and c_i:c_a

V_{cmax}, J_{max} and R_{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_{dark} and V_{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 CO_2 between c_a and c_i. However, this interpretation assumes that a higher V_{cmax} means a higher assimilation rate – which cannot be true if V_{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_{cmax}, J_{max} and R_{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_{dark} and V_{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_{cmax} and J_{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_{cmax} and J_{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_{cmax} and J_{max} depending on antecedent environmental conditions. Field measurements of photosynthetic parameters in different seasons are required to test these predictions (Bloomfield et al., 2018).

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.

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_{cmax}, J_{max} and R_{dark} and the least-cost hypothesis for c_i:c_a.

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

Figures
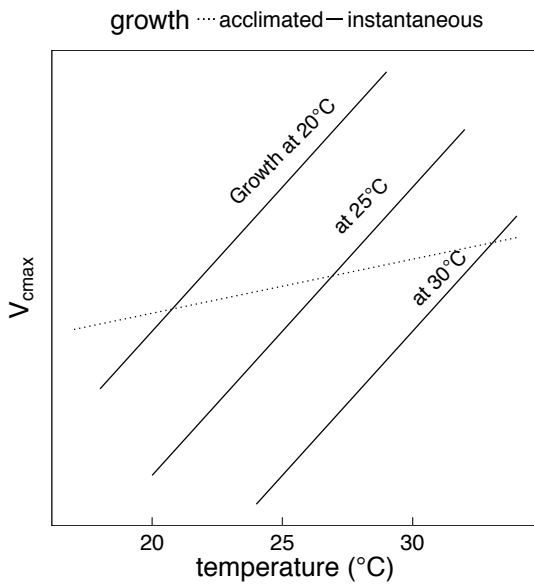
Figure 1: Schematic showing the difference between instantaneous and acclimated V_{cmax} as predicted by the coordination hypothesis. The three solid lines indicate the instantaneous response of V_{cmax} to temperature for plants grown at 20˚C, 25˚C and 30˚C under identical light conditions. The dashed line indicates the predicted response of field-measured (acclimated) V_{cmax} to the growth temperature. The instantaneous responses are due to enzyme kinetics, whereas the acclimated response is due to the slightly higher V_{cmax} required to compensate for increased photorespiration at higher temperatures. Because the acclimated response is shallower than the instantaneous response, V_{cmax} at a common temperature (e.g. 25˚C) declines with growth temperature.
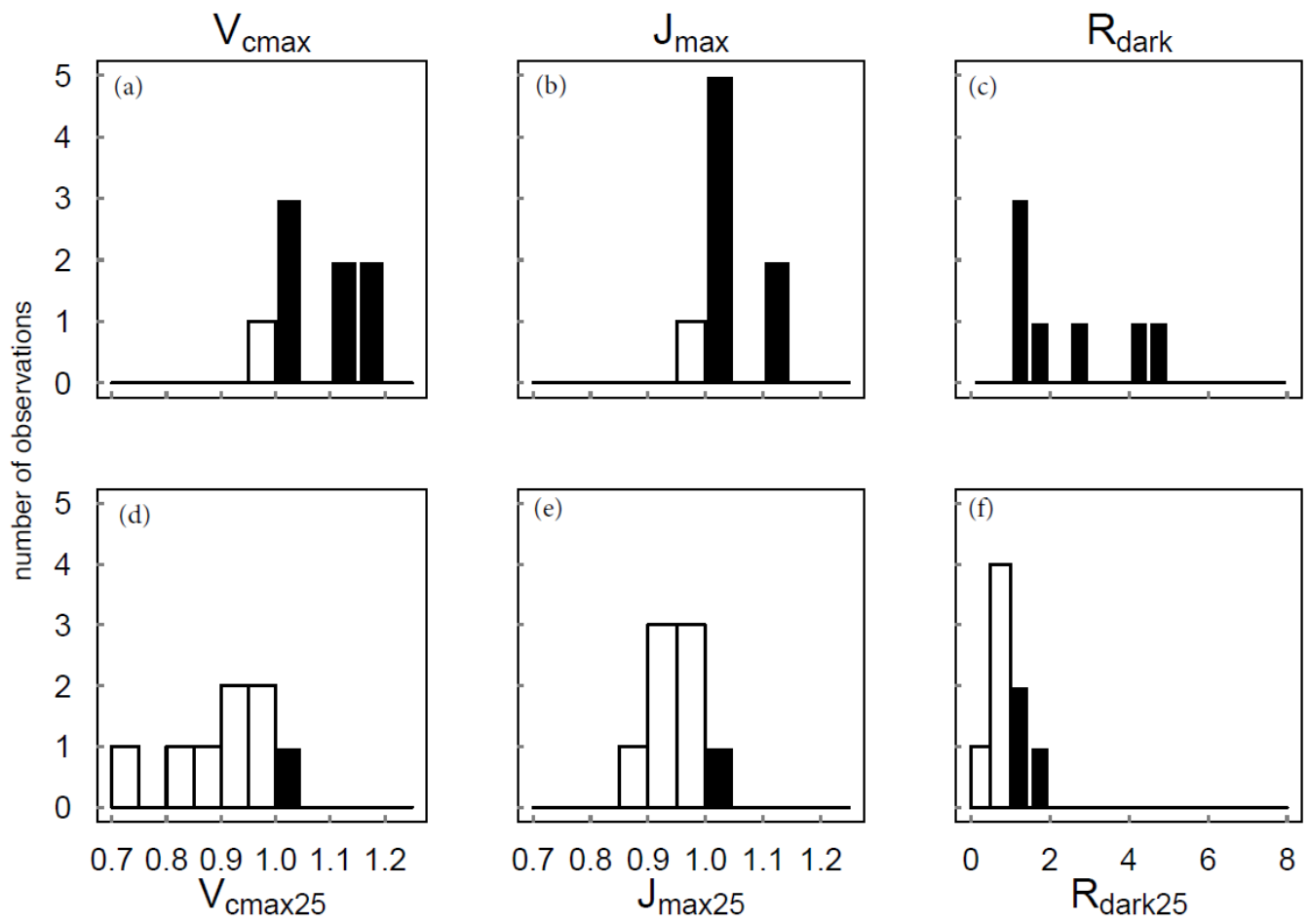
Figure 2: Distributions across species of the warm:cool season ratios of V_{cmax}, J_{max} and R_{dark} measured at ambient temperature (top panels) and standardized to 25°C (bottom panels). Ratios > 1 are shown as black bars, ratios < 1 as white bars ($n = 8$).
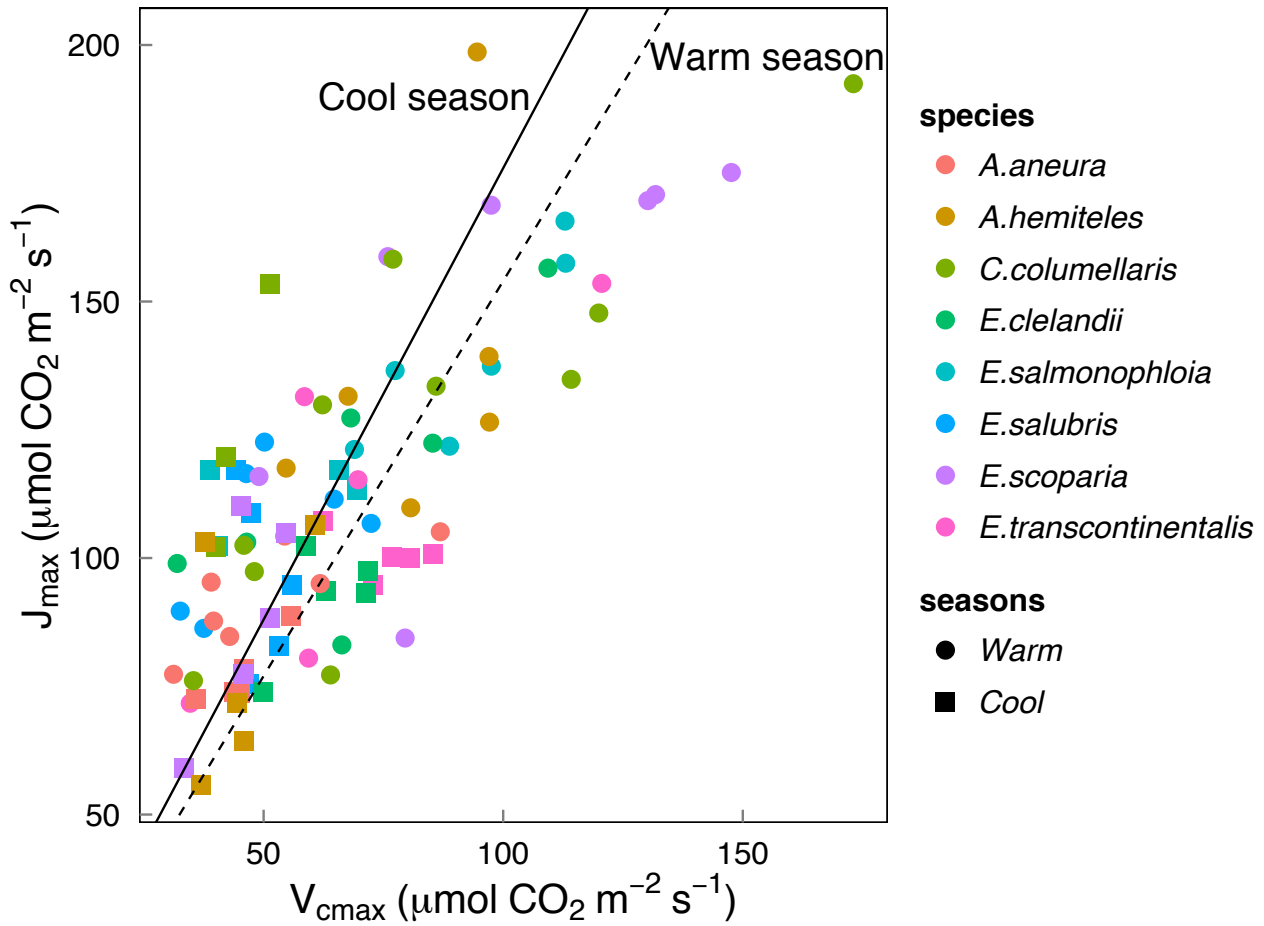
Figure 3: Linear regressions forced through the origin between J_{max} (μmol m^{-2} s^{-1}) and V_{cmax} (μmol m^{-2} s^{-1}) for individual trees of eight species in the warm season (circles, dashed line, slope = 1.58) and the cool season (squares, solid line, slope = 1.79). Both regressions are significant ($p < 0.05$). Each point represents one A-c_i curve ($n = 86$).
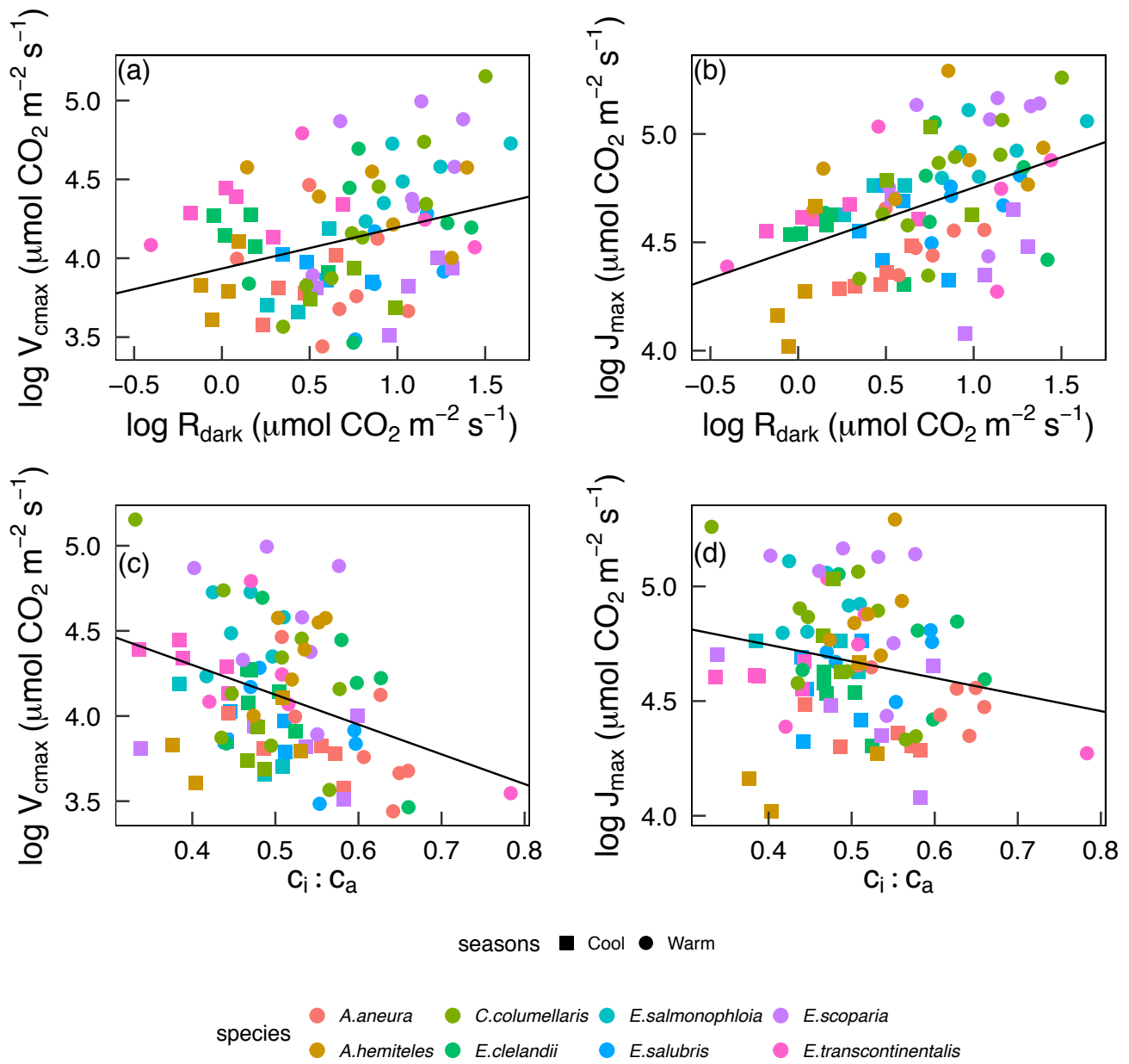
Figure 4: Linear regressions of individual trees by season (n=16) and species (n=8) for photosynthetic capacity, V_{cmax} and J_{max} with R_{dark} (μmol m^{-2}s^{-1}) and the c_i:c_a ratio at ambient $CO_2 \approx 400$ μmol mol^{-1} ($p < 0.05$). V_{cmax}, J_{max} and R_{dark} were log$_{ln}$ transformed; c_i:c_a was not. Each point represents one A-c_i curve (n=86).
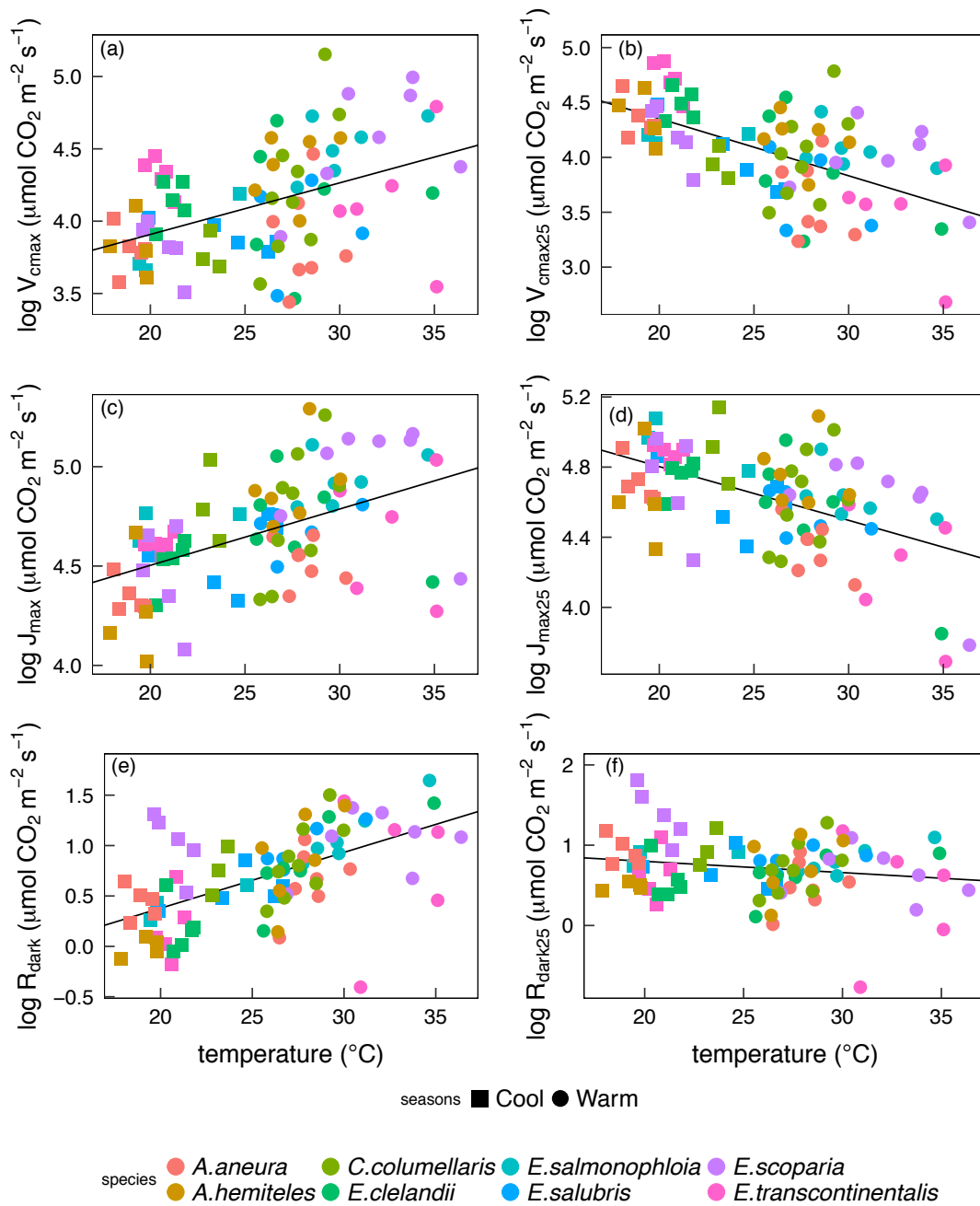
Figure 5: Bivariate linear regressions ($p < 0.05$) of natural log-transformed V_{cmax}, V_{cmax25}, J_{max}, J_{max25}, R_{dark} and R_{dark25} (μmol m^{-2} s^{-1}) *versus* leaf temperature (T_{leaf}, °C). Each point represents one A-c_i curve ($n = 86$).
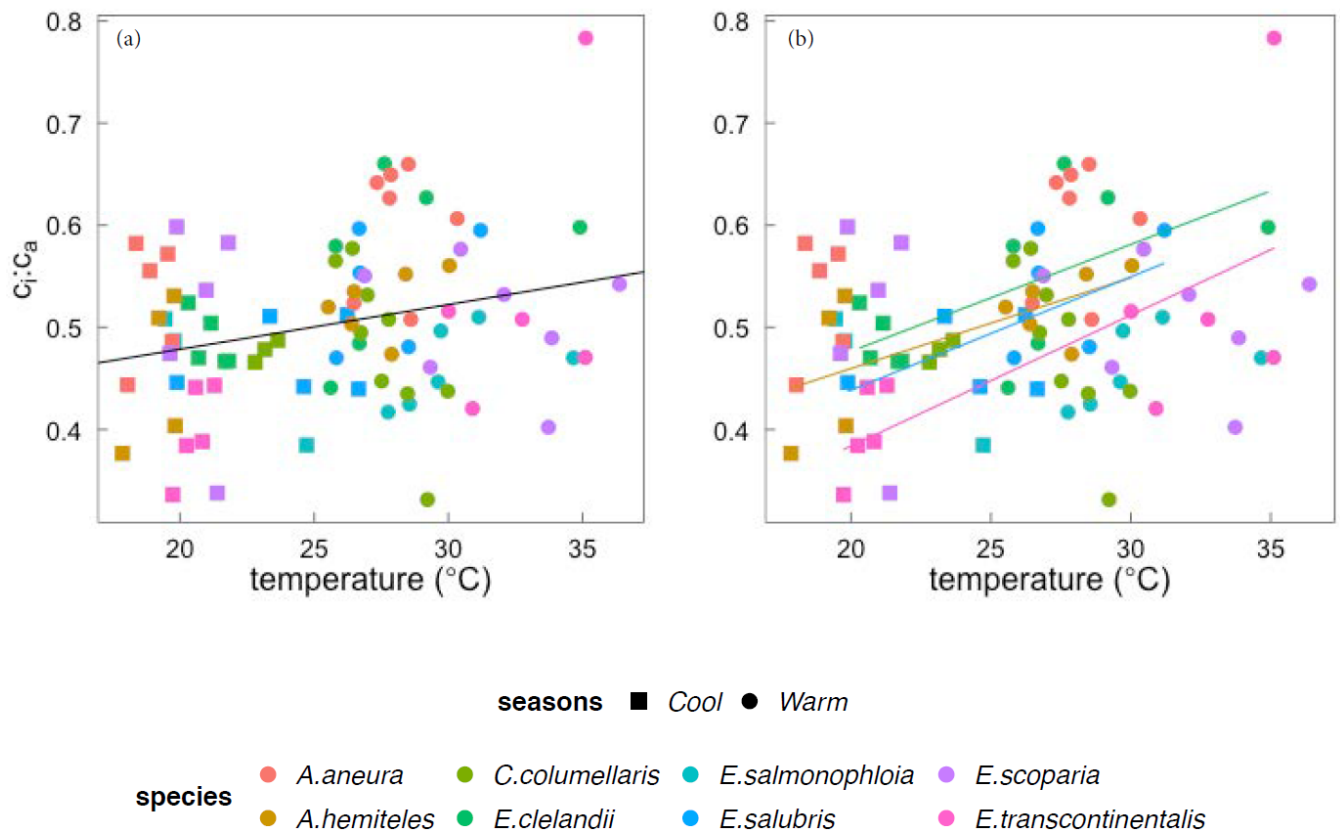
Figure 6: Bivariate linear regressions of the c_i:c_a ratio (at ambient $CO_2 \approx 400$ µmol mol^{-1}) *versus* temperature (T_{leaf}, °C) for individual trees considering all data (a) and within species (b). Only significant regressions ($p < 0.05$) are shown. Each point represents one A-c_i curve ($n = 86$).
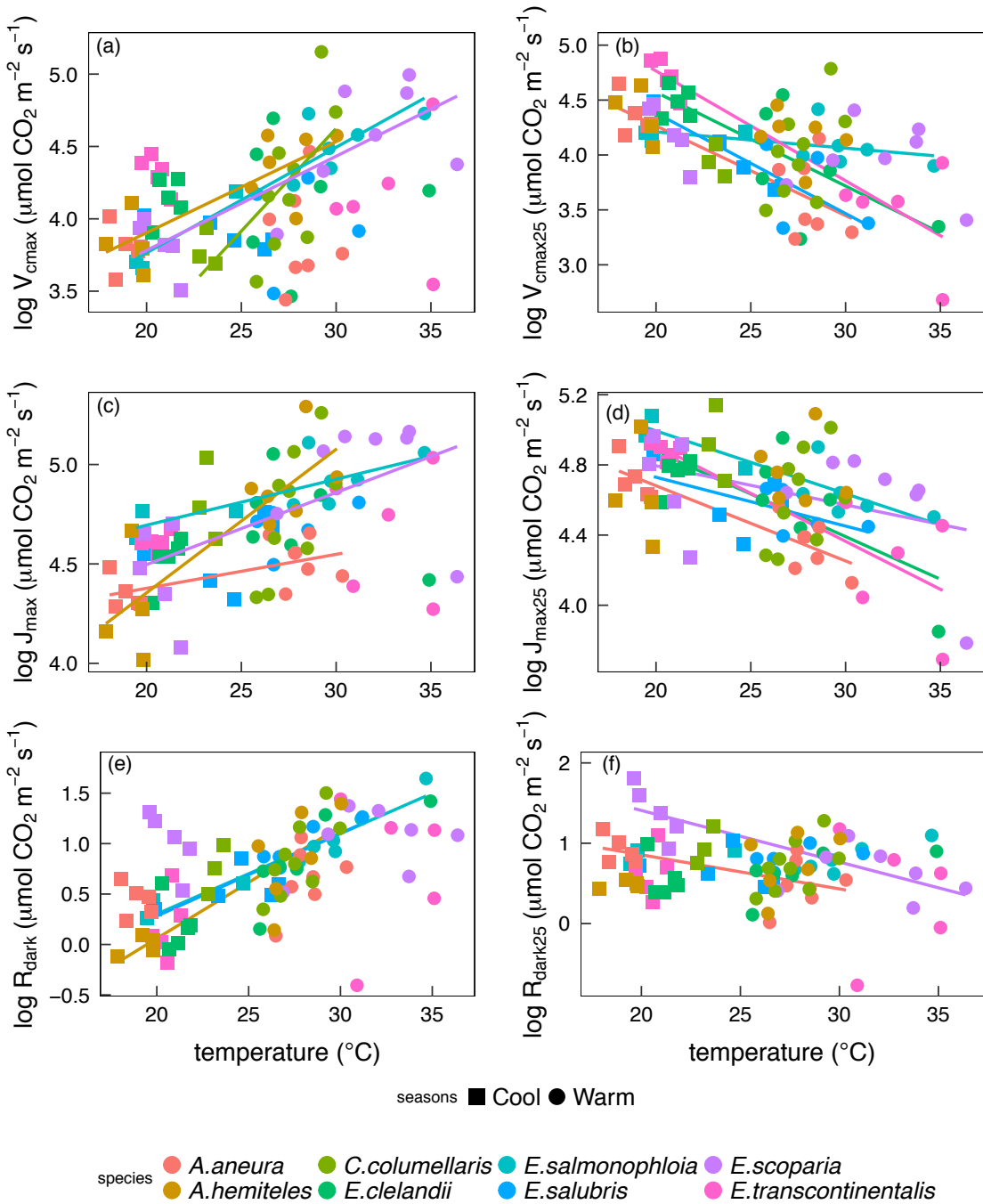
Figure 7: Bivariate linear regressions of natural log-transformed V_{cmax}, V_{cmax25}, J_{max}, J_{max25}, R_{dark} and R_{dark25} (μmol m^{-2} s^{-1}) *versus* leaf temperature (T_{leaf}, $^{\circ}$C) within species ($p < 0.05$). Only significant regressions ($p < 0.05$) are shown. Each point represents one A-c_i curve ($n = 86$).
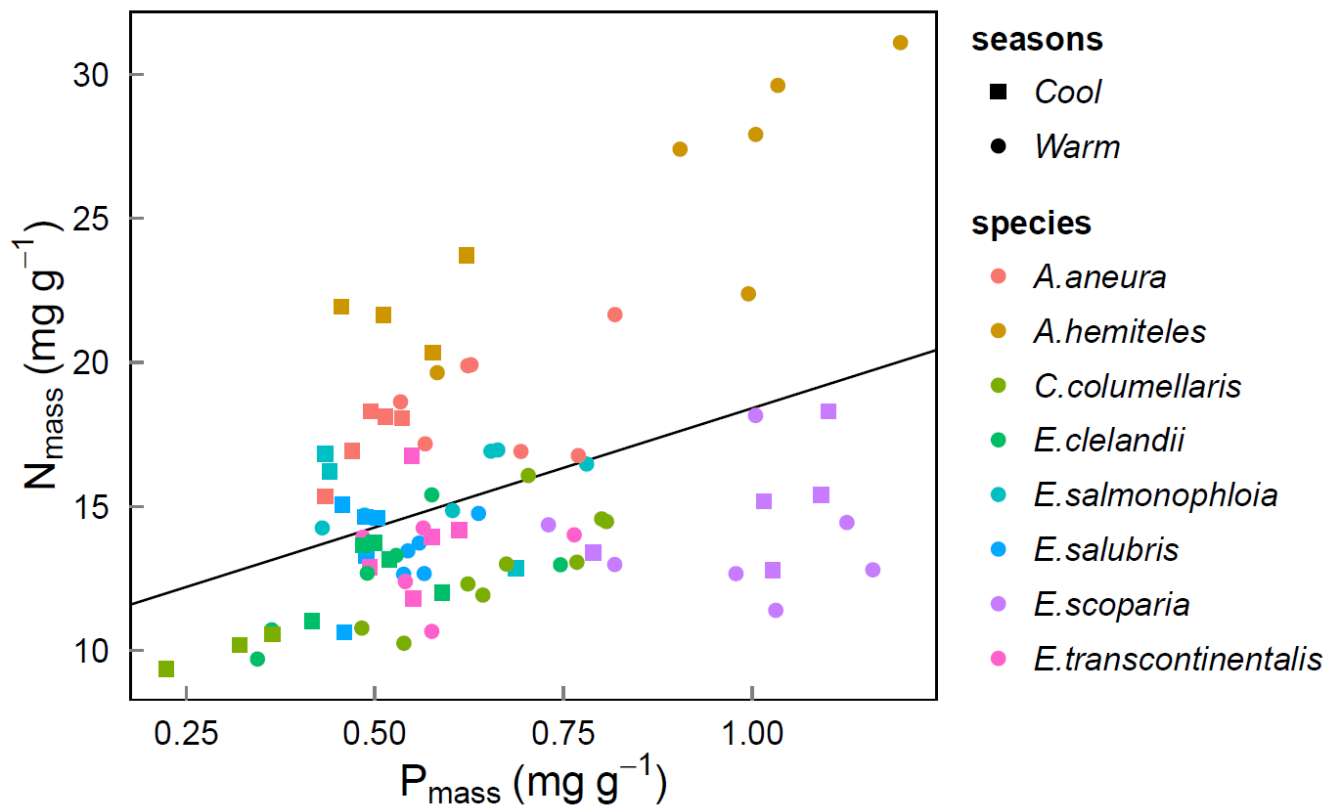
Figure 8: Bivariate linear regression of N_{mass} (mg g^{-1}) *versus* P_{mass} (mg g^{-1}) for all data (black line, slope = 0.33, R^2 = 0.17). Each point represents one leaf (n = 86).
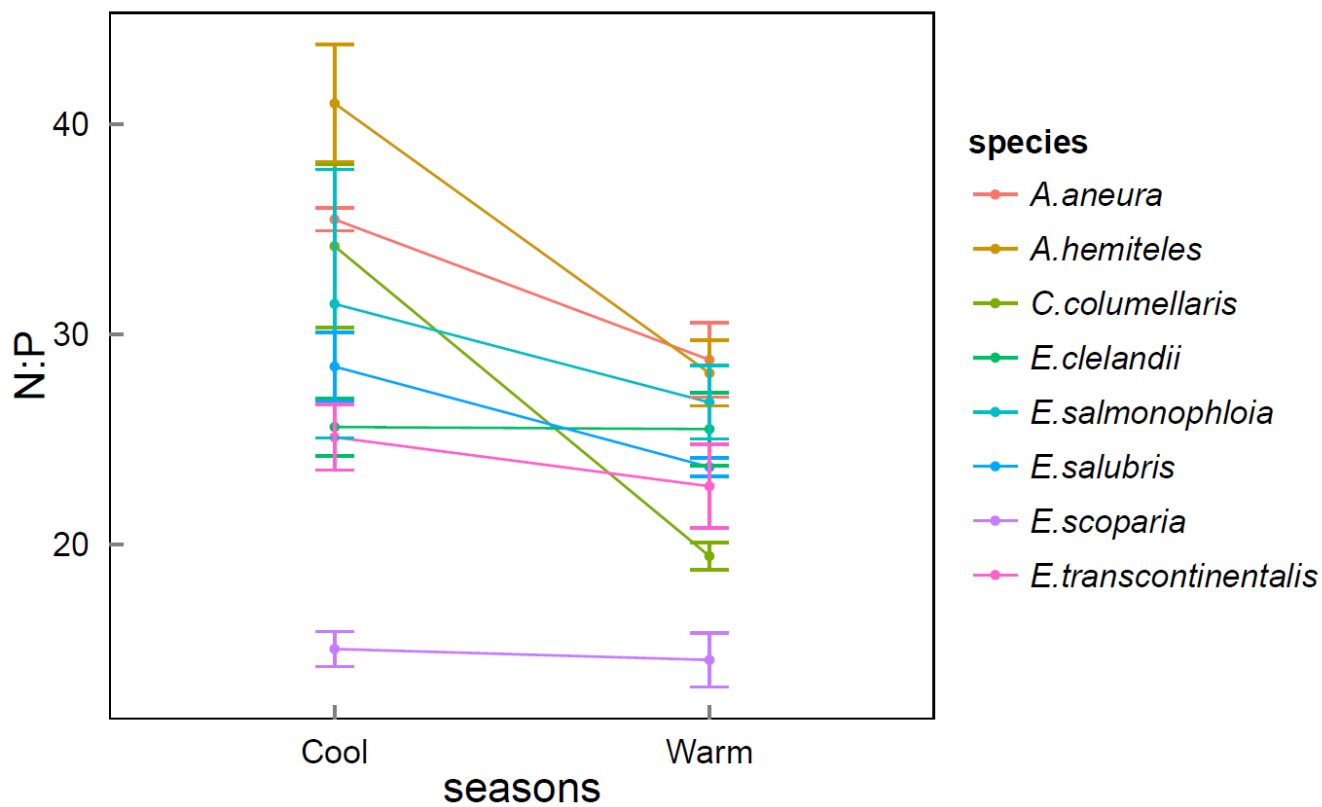
Figure 9: Changes in the average foliar N:P ratio for each species between the cool and the warm seasons. Standard errors shown.