

Interactive comment on “Thermal acclimation of leaf photosynthetic traits in an evergreen woodland, consistent with the co-ordination hypothesis” by Henrique Fürstenau Togashi et al.

Henrique Fürstenau Togashi et al.

henriquetogashi@gmail.com

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We thank the reviewers for comments and suggestions that have helped to improve our manuscript. Our response is organised 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.

C1

- We have moved the equations to the main text and provided further discussion there. This discussion now includes the rationale for the linear assumptions.

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 5 and 6. Only significant individual lines ($p < 0.05$) are shown in Figures 5 and 6, as is described in the Figure captions and in the Results section (3.1 and 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.

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 reviewer makes an insightful point here. The key evidence is the change in

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V_{cmax}. It is not completely clear what the co-ordination hypothesis predicts about J_{max}. The reviewer may be right that “it is generally taken” that the co-ordination hypothesis is all about the partitioning of leaf N to J_{max} versus V_{cmax}. But the co-ordination hypothesis merely states that the two limiting rates of photosynthesis tend to be equal under average conditions. Limitation by J_{max} is not normally reached under natural conditions; at low light photosynthesis is limited by J (not J_{max}) and at high light it becomes limited by V_{cmax}. So the first-order prediction of the co-ordination hypothesis is that V_{cmax} should acclimate to the average light conditions. A subsidiary hypothesis is then required to predict the ratio of J_{max} to V_{cmax} (Wang et al., 2017). We hope we have now made this clear in the text, while avoiding too much distracting complexity.

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 generalised 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.

AR1.7. Also, the fitted slopes for all parameters are calculated as log₁₀(parameter)

C3

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.

- The data presented has now being standardise to ln.

AR1.8. 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 entirely agree with this suggestion. The co-ordination hypothesis predicts that V_{cmax} should acclimate so as to just use the available light under average conditions (as pointed out above) and that implies (as pointed out by Maire et al., 2012) that there is an optimal leaf N for any given set of average environmental conditions. It is not just a hypothesis about the allocation of a given amount of N to different functions within the leaf. 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 attached.

AR1.9. 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

C4

photosynthetic co-limitation is needed.

- The reviewer is correct to indicate that (as with J_{max}) 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 has been clarified.

AR1.10. 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 to cover these points.

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 included the suggested citations, and commented on the issue raised.

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

C5

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 brief comment on this.

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) $\text{mol m}^{-2} \text{s}^{-1}$ for the warm season, and (0.057 – 0.078 – 0.085) $\text{mol m}^{-2} \text{s}^{-1}$ for the cool season. We have added a note on this point.

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_{cmax} and J_{max} values against leaf temperature.

- The real 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.

Technical comments

C6

AR2.7. Page 2, Line 13 - please clarify what "these" refers to - V_{cmax} and J_{max} , yes?

- Yes. We have clarified this now.

AR2.8. Page 9, Line 7 - the relationship between C_i/C_a and photosynthetic capacity could also be because higher photosynthetic capacity (at a constant g_s) reduces C_i . Cause and effect can't be determined.

- We have added a note on this alternative explanation.

AR2.9. If all the gas exchange is determined with a Licor IRGA, how are the parameters being reported in units of electrons and O_2 ? J_{max} and R_{dark} should be in units of CO_2 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 a 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 second, we sampled the youngest fully expanded leaves in both seasons. We have added a comment in the text to this effect.

AR3.2. Equation 1 is used to estimate V_{cmax} and J_{max} at 25C. During the warm

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period (unclear time of day A- C_i 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- c_i 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 some 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).

C8

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.

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.

- We have now provided information in the text 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)

C9

- We have now explained what we are doing here in the revised text. It is certainly a simplification – see our response to AR1.4 above.

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

- We have changed the wording here to be more explicit.

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, and T_{air} is the air temperature. We have noted this in the methods section.

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 eight 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 include 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! We have added some words to clarify this further.

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.

C10

- 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.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-449>, 2017.

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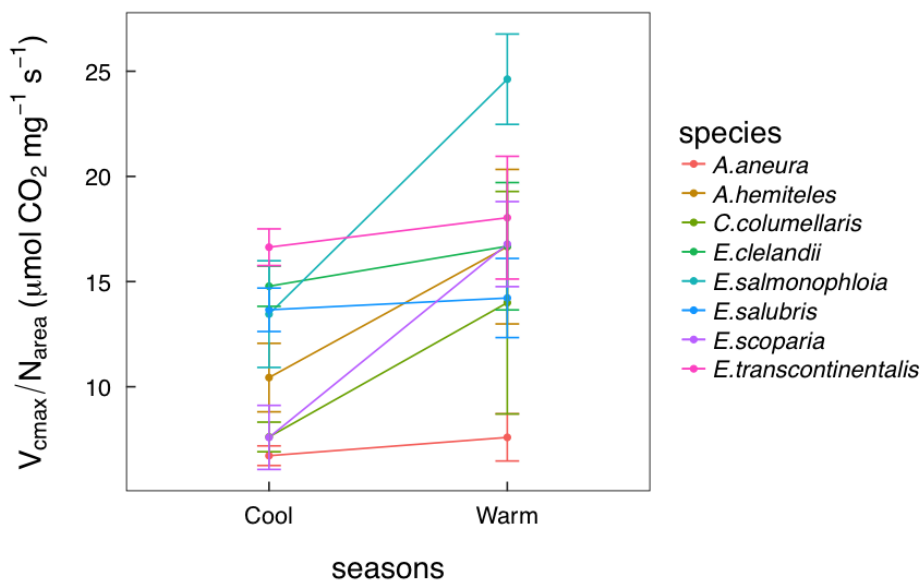


Fig. 1.

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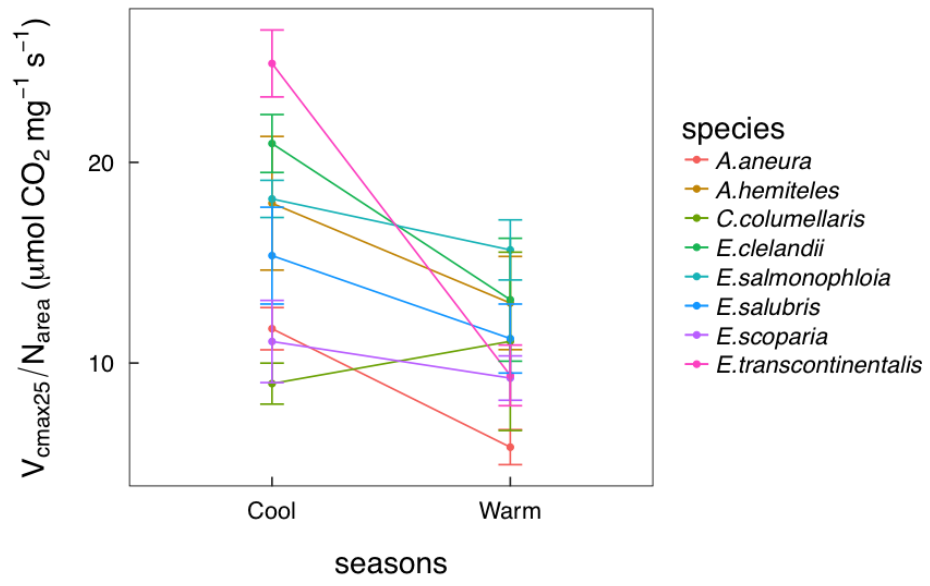


Fig. 2.