Dear referee

Thank you very much for your review of my paper entitled “C_3 plants converge on a universal relationship between leaf maximum carboxylation rate and chlorophyll content”. We appreciate the careful and valuable comments and suggestions from you and we have revised the manuscript accordingly.

The point-by-point responses to comments were listed below. The major changes in the manuscript were attached in red for the convenience of the reviewers. All the changes in the revised manuscript were highlighted in yellow.

Best regards,

Liangyun Liu

Response to the referee

1. My primary concern about the manuscript has to do with the claim of ’universality’, which probably comes with little surprise. To start, the number of species considered by their analysis is quite limited. For trees, their dataset only considers four temperate broadleaf species, of which two (P. grandidentata and P. tremuloides) are incredibly closely related. What about gymnosperms? Does this hold for douglas fir and pinyon pine? Or evergreen oaks? Various C3 grasses? Arid shrubs? Any claim to universality must have a far more diverse collection of underlying species considered by the study.

The claim to universality is further undercut by the actual amount of data collected per species. Some of the shrub and vegetable species have fewer than ten measurements. The discrepancy is visually highlighted in Figure 4, where there are only a handful of ’vegetable’ and ’shrub’ data points. As a result, the overarching relationships are driven by the vastly larger "(Temperate Broadleaf) Tree" and "Crop" datasets. This discrepancy actually goes to undercut the overall message, as the slope of the Vcmaxchl relationship definitely doesn’t look the same for the vegetable data (NRMSE ~ 50 percent). Furthermore, both these datasets seem to have been previously published to highlight the strength of the Vcmax-chlorophyll relationship (Croft et al 2017; Qian et al 2019). While it’s fine to combine previously published datasets to derive new insights, the authors here could do a better job of framing how the combination datasets allows for a new advance. As presently constructed, the manuscript implicitly suggests that the strength of the Vcmax-Chl relationship is a mostly novel finding.

Response:
We take the reviewer’s point; a claim of ‘universality’ in the Chl-Vcmax relationships should probably consider more plant species. To support an investigation of the robustness of the Chl-Vcmax relationship we did also in additional sources of data from aligned measurements (Vcmax, Jmax, Chl) from a greater number of species from the literature. Unfortunately, most Chl data was inferred from leaf N relationship not measured data. The lack of available paired Chl-Vcmax measurements generally in the literature prevented us from including an additional species in the direct analysis.

However, we do collected more in-situ Chl-Vcmax measurements, which includes crops, trees, shrubs and vegetables in different environmental conditions. These data show that chlorophyll and Vcmax do have a convergent relationship across different plant types. Moreover, the relationships between Vcmax, Jmax and chlorophyll on more species from other literatures in the discussion section indirectly verify the relationship between Vcmax and chlorophyll. We recognized that more experiments are need to further validate the relationship. After careful consideration, we remove the word 'universal' from the title to avoid over-reaching. Some relevant descriptions were also changed in the manuscript.

Revisions:

Title

C_3 plants converge on a relationship between leaf maximum carboxylation rate and chlorophyll content

1 Introduction

…This should help to provide an operational approach for the retrieval of $V_{cmax,25}$ using leaf chlorophyll content across different plant types.

2.1 Study sites and samples

…We sought to include as many species and plant functional types as possible in the dataset,…

4.3 Chlorophyll–$V_{cmax,25}$ relationships

Approach 2: Direct relationships between V_{cmax}, J_{max} and chlorophyll

(3) Relationships between V_{cmax} and chlorophyll

…Therefore, it can help to map $V_{cmax,25}$ using satellite remote sensing data based on the relationship between leaf $V_{cmax,25}$ and chlorophyll content.

5 Conclusions

…However, more data are need to further validate the relationship. It can be seen that leaf chlorophyll has the potential for use as a proxy for $V_{cmax,25}$. These findings can help to estimate leaf $V_{cmax,25}$ via its relationship with chlorophyll content, which can be retrieved from satellite remote sensing data.

2. I was wondering if the authors might not do a little more work to expand their dataset further still. Table 2 lists a number of previous studies that have explored the Vcmax-Chl relationship. Have the authors considered combining their data with the Vcmax-chl data they have collected? It could be interesting to more thoroughly and exhaustively combine datasets in a statistical framework to understand how things like phylogeny (species/genus), leaf habit (evergreen/broadleaf) and anigo/gymnosperm affect the Vcmax-chl relationship. My suspicion is that the results would point toward a fairly consistent Vcmax-chl relationship, but would do so in a framework that more holistically appreciates the wide array of C3 plant types.

Response:

Table 2 lists a number of previous studies that have explored the Vcmax-Jmax relationship. However, they did not have measured Chl data or Chl data that matched the Vcmax. We tried to find the measured Chl and Vcmax in other literatures to expand our dataset. In Houborg et al. (2015), the relationships between Vmax and Chl relationships were established based on N versus Chl and Rubisco versus N regression. In Luo et al. (2019), Vcmax-Chl regression relationships are partly derived from the literature (Croft et al., 2017), and some are derived from the derivation based on N. In Alton (2018), the Chl data was inferred from measured leaf N relationship. There are surprisingly few studies that have measured co-incident Chl and Vcmax values. As mentioned, it has always been N that is the biochemical measurement paired to Vcmax.

In Bahar et al. (2017), measurements were made on the leaves attached to the cut branches. The leaves cannot avoid wilting under the condition of in vitro measurement although the cut branches were put into water. In fact, we had the similar experiments. In August 2018, we made some measurements on white birch in Daxinganling in northeastern China. For the convenience of operation, we cut the branches, took them back to the laboratory immediately and put them into buckets filled with water for measurement. Unfortunately, the leaves still showed a certain degree of water loss which affects photosynthesis. As shown in Figure S1, red line is the regression relationship between leaf $Vcmax_{25}$ and chlorophyll measured in vivo, while the measured values of $Vcmax_{25}$ in vitro are lower than those measured in vivo overall. Therefore, these data were not combined into our dataset.

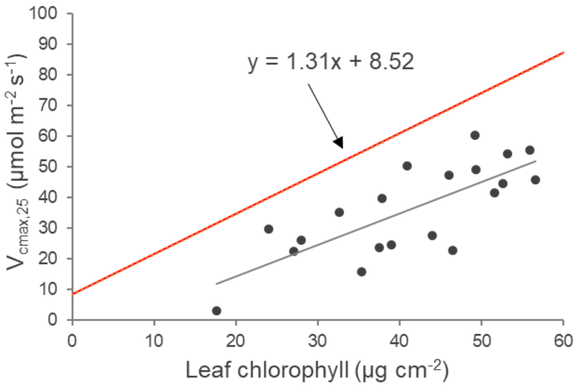

Figure S1. The relationship between leaf $V_{cmax,25}$ and chlorophyll measured in vitro. Red line is the regression relationship measured in vivo. Black point is the values measured in vitro.

3. The authors also some logical jumps that weaken their overall argument. The first is relatively minor. In the introduction the authors indicate that the consistency of the Chl:Vcmax relationship is stronger than the N:Vcmax relationship and use this as the basis for focusin on Chl in the main body of the text. I was hoping that the authors would revisit this claim in their analysis. It would be nice to establish that the Chl:Vcmax relationship is i) strong and ii) stronger than alternatives. Again, this is a relatively minor point, but one that would make any claim to universality much more convincing.

Response:
We have added the comparison of the relationships between leaf $Vcmax_{25}$ with both chlorophyll and nitrogen content in the first paragraph of the discussion section.

Revisions:
4.1 Comparison of the relationships between leaf $V_{cmax,25}$ with both chlorophyll and nitrogen

content

As shown in Fig. 5, the cotton and tree samples that had co-incident leaf nitrogen data available, were used to investigate the relationships between leaf $V_{cmax,25}$ with both chlorophyll and nitrogen content. The results show a strong relationship between leaf $V_{cmax,25}$ and chlorophyll content (R^2 = 0.74). However, the relationship between leaf $V_{cmax,25}$ and nitrogen content was weaker (R^2 = 0.33). This weak relationship may further prove the need for deriving function specific nitrogen fractions rather than total nitrogen for modelling leaf $V_{cmax,25}$. Furthermore, the relationships between leaf $V_{cmax,25}$ and nitrogen content are not well consistent across species. Consequently, these results demonstrate that the relationship between leaf $V_{cmax,25}$ and chlorophyll content is strong and stronger than that between leaf $V_{cmax,25}$ and nitrogen content.

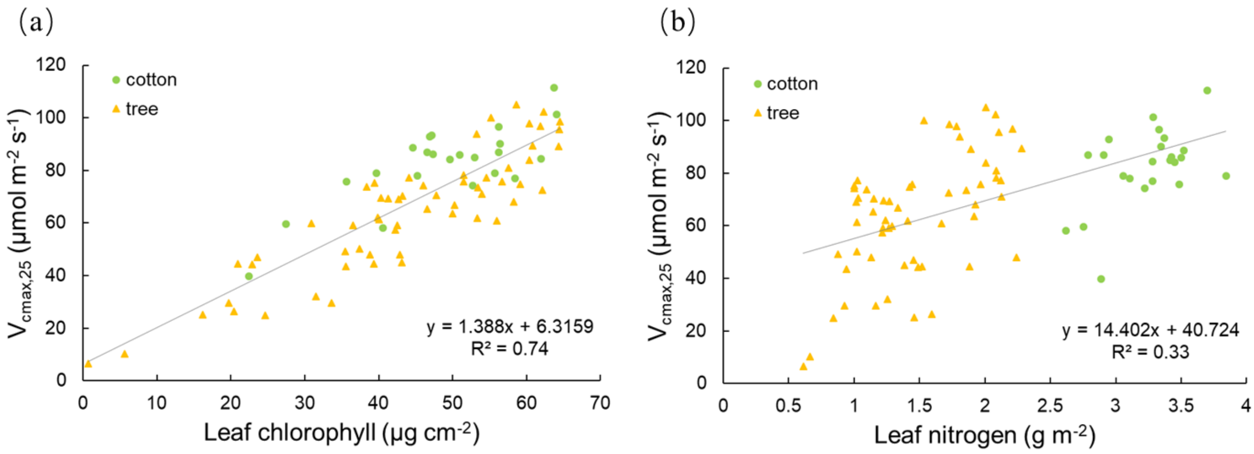

Figure 5. Relationships between leaf $V_{cmax,25}$ and (a) chlorophyll; (b) nitrogen for cotton in 2017 and trees in 2014.

4. The second logical jump is slightly more important. Throughout the introduction, discussion, and conclusion that authors make the repeated claim that establishing a strong Chl:Vcmax relationship might enable mapping Vcmax at the global scale using remote sensing. This is a huge jump. From my reading, the data analyzed here is leaf level data. Satellites see canopies – not leaves. How sure are we that leaf level relationships hold at the canopy scale? I am especially reminded of the back and forth between Ollinger et al. 2008 and Knyazikhin et al 2013, both published in PNAS. Ollinger put forth an approach for measuring canopy nitrogen content, while Knyazikhin argued those "spectra-nitrogen" relationships were more than likely driven entirely by variations in canopy structure. How likely are the critiques of Knyazikhin to apply to remote sensing measurements of chlorophyll? Certainly I do not expect the authors to have all the answers to questions like these, but it seems inappropriate to ignore such concerns entirely. Reliably estimating just about anything from remote sensing requires consistency (in a physical sense) between what satellites measure and what we measure on the ground. In my mind, the remote sensing world has repeatedly undercut its credibility by avoiding, as opposed to embracing, issues of scale.

Response:

This paper focuses on estimating leaf $Vcmax_{25}$ from leaf chlorophyll content. In fact, we are getting very close to a global leaf-level chlorophyll map. Currently Dr. Croft (co-author) has a paper in revision in Remote Sensing of Environment (‘The Global Distribution of Leaf Chlorophyll Content’) using MERIS data to produce global maps (300 m resolution), every 7 days. The approached using radiative transfer models to separate out the contribution from canopy properties (LAI, leaf architecture, tree density etc.) and background reflectance, to model leaf-level reflectance, from which we can relatively straightforwardly derive leaf chlorophyll content. This is approach is outlined in previous papers (Croft et al., 2013, Remote Sensing of Environment, 133: 128-140) We have added some text about the retrieval of leaf chlorophyll content from canopy spectra in the introduction section.

Revisions:

1 Introduction

…Secondly, the spectral bands that correspond to leaf nitrogen content are influenced by atmospheric water vapour, foliar water content and cellular structure scattering (Herrmann et al., 2010), making it difficult to accurately derive leaf nitrogen content from satellite observation data. However, it has become possible to retrieve leaf chlorophyll from canopy spectra. Luo et al. (2019) mapped global leaf chlorophyll content from the MEdium Resolution Imaging Spectrometer (MERIS) surface reflectance using a two-step process-based algorithm (Croft et al., 2013). Xu et al. (2019) retrieved leaf chlorophyll content using a matrix-based vegetation index combination approach from Sentinel-2 data. Jay et al. (2017) estimated leaf chlorophyll content in sugar beet canopies using images from hyperspectral camera. Moreover, a number of vegetation indices, such as MTCI (Dash and Curran, 2004), TCARI/OSAVI (Rondeaux et al., 1996;Daughtry et al., 2000), and ND_{705} (Gitelson and Merzlyak, 1994) are widely used to retrieve leaf chlorophyll content from remotely sensed data. In this case, an alternative approach to estimating $V_{cmax,25}$ is through the use of leaf chlorophyll content, which is much more accurately derived from remote sensing techniques due to its well-defined absorption features at visible wavelengths (Croft and Chen, 2017).

5. I will make a final note concerning citations. Overall, the citations tend to skew toward the more recent (e.g., 2017 or newer). There were also several instances where the authors cite a paper that discusses a topic (e.g., L160 citing Croft et al 2017 in reference to b6f/NADPH), as opposed to citing a more direct paper that focuses on the topic. I would encourage the authors to careful revisit their citations to make sure the appropriate literature is cited.

Response:

Thank you for your comment. We have checked the citations to make sure the appropriate literature is cited. The sentence presented by the reviewer has been removed from the revised manuscript.

Revisions:

1 Introduction

…In most classical biochemical models, $V_{cmax,25}$ is usually hypothesized to be a fixed value for a given plant functional type (Wullschleger, 1993;Medlyn et al., 1999;Oleson et al., 2010;Rogers et al., 2017)…

2.2 Estimation of leaf chlorophyll and nitrogen content

…Foliar chlorophyll was extracted using spectra-analysed grade *N*, *N*-dimethylformamide, and the absorbance was measured using a Shimadzu UV-1700 spectrophotometer (Wellburn, 1994). Nitrogen content was also measured during the 2014 season. Leaf samples were dried at 80°C for 48 h, ground to a powder using a Wiley mill and analysed on an ECS 4010 Elemental Combustion System for CHNS-O analysis (Costech Analytical Technologies, Valencia, California) (Croft et al., 2017).

4.2 Physiological basis for the relationships between leaf $V_{cmax,25}$ and chlorophyll content

The results in this study demonstrate that leaf chlorophyll content can be used to model $V_{cmax,25}$ directly. The study attempts to elucidate the physiological mechanism that the direct use of chlorophyll by the relationships between leaf V_{cmax}, J_{max} and chlorophyll content. Adjusting the concentration of leaf chlorophyll pigments is one of the most effective mechanisms by which plants regulate light absorption. Leaf chlorophyll is related to the photosynthesis rate because of its decisive role in the instantaneous electron transport rate (Porcar-Castell et al., 2014). Therefore, a limitation on electron transport occurs when the number of quanta absorbed is insufficient. That is to say, the electron transport rate depends on the incident photosynthetically active radiation (PAR) and the efficiency of the light-harvesting complex. Theoretically, J_{max} is related to leaf chlorophyll content. Experimentally, the regeneration capability of Ribulose 1,5-bisphosphate (RuBP) increases linearly with total leaf chlorophyll content (Singsaas et al., 2004). Studies have also suggested a linear relationship between leaf $J_{max,25}$ and chlorophyll content (Nolan and Smillie, 1976;Ripullone et al., 2003;Warren et al., 2015;Alton, 2017). Jmax is related to the ability to transport electrons to produce ATP and NADPH, which are then used to drive the carbon reactions by reducing Rubisco into RUBP. In principle, it takes around two electrons to consume one unit of Rubisco on average, which implies a constant ratio between $J_{max,25}$ and $V_{cmax,25}$ (Luo et al., 2018). A quasi-linear relationship is measured between $J_{max,25}$ and $V_{cmax,25}$ (Wullschleger, 1993;Meir et al., 2002;Kattge et al., 2009;Walker et al., 2014). V_{cmax} and J_{max} have also been shown to be tightly coupled, and the ratio of $J_{max,25}$ to $V_{cmax,25}$ is typically assumed to have a fixed value in terrestrial biosphere models (Wohlfahrt et al., 1999;Leuning, 2002;Medlyn et al., 2002;Kattge and Knorr, 2007). Based on the above theories and assumptions, it is reasonable to suggest that there is a mechanistic basic to the relationship between leaf $V_{cmax,25}$ and the chlorophyll content.

6. L50 inevitably? This confused me

Response:
We have changed the sentence as follows

Revisions:

1 Introduction

As a key parameter in photosynthesis, $V_{cmax,25}$ is likely to be correlated with plant functional traits (Serbin et al., 2012;Croft et al., 2017;Smith et al., 2019)…

7. L166: "Adjusting the concentration of leaf chlorophyll pigments is one of the most effective mechanisms by which plants regulate light absorption." Such a claim could use a citation. This is certainly an interesting point, but I am not familiar with the literature that supports this line of argument.

Response:

This argument and the next argument are from the same reference, so we only marked the citation after the second argument. The original text in Porcar-Castell et al. (2014) is "Accordingly, an effective mechanism used by plants to regulate light absorption, or fAPAR, consists of adjusting the concentration of chlorophyll pigments in the leaf."

8. Why do the authors use NRMSE? One of the main reasons to use RMSE is that it has units that make sense. Given that all the analyses are in terms of Vcmax, it seems more informative to use RMSE.

Response:
Normalizing the RMSE facilitates the comparison between datasets or models with different scales. The number of samples of each type in Figure 4 is inconsistent. Therefore, we kept the NRMSE, and added the RMSE. We have made relevant modifications in Table 3, Figure 4 and the manuscript.

Revisions:

Abstract

…Validation showed that the model performs well, producing relatively low root mean square errors (RMSE) for crops, shrubs, trees and vegetables (RMSE = 16.53, 18.98, 12.06 and 19.11μmol m^{-2} s^{-1}, respectively) …

3.3 Relationships between leaf $V_{cmax,25}$ and chlorophyll content

…The model performs well, giving relatively low root mean square errors (RMSE = 16.53, 18.98, 12.06 and 19.11 μmol m^{-2} s^{-1} for crops, shrubs, trees and vegetables, respectively) …

4.3 Chlorophyll-$V_{cmax,25}$ relationships

Approach 1: Semi-mechanistic model using nitrogen as an intermediary

…

We tested the above approach using different plant types. Except for the vegetable samples, the estimated values were found to be higher than the measured values, with a bias of 12.81, 18.54 and 5.13 μmol m^{-2} s^{-1} for crops, shrubs and trees, respectively. The RMSE values (23.32, 31.08, 17.67 and 20.68 μmol m^{-2} s^{-1} for crops, shrubs, trees and vegetables, respectively) were slightly higher, indicating relatively low accuracy compared to our results for C_3 plants. Therefore, using nitrogen as an intermediary to establish a stable relationship between leaf $V_{cmax,25}$ and chlorophyll content across different C_3 plants may introduce some bias.

Approach 2: Direct relationships between V_{cmax}, J_{max} and chlorophyll

（3）Relationships between V_{cmax} and chlorophyll

…All four models were found to have similar slope parameters ranging from 1.11 to 1.70, with RMSE values < 18 μmol m^{-2} s^{-1}….

5 Conclusions

…A linear empirical model was built to retrieve leaf $V_{cmax,25}$ from chlorophyll content for different plant types, with good validation results between estimated and measured $V_{cmax,25}$ (RMSE = 16.53, 18.98, 12.06 and 19.11μmol m^{-2} s^{-1} for crops, shrubs, trees and vegetables, respectively) ...

Table 3. Statistics corresponding to the comparison with measured $V_{cmax,25}$ for the several combinations considered. Positive (negative) bias indicates overestimation (underestimation) by the models compared to measured values.

Model	Combination	Formula	RMSE(μmol m^{-2} s^{-1})	NRMSE	Bias
Model1	Equations (9) and (11)	$V_{cmax} = 1.46 \times Chl - 3.11$	16.21	0.13	-4.56
Model2	Equations (9) and (12)	$V_{cmax} = 1.11 \times Chl + 10.89$	17.05	0.14	-6.70
Model3	Equations (10) and (11)	$V_{cmax} = 1.70 \times Chl - 6.49$	16.91	0.13	3.13
Model4	Equations (10) and (12)	$V_{cmax} = 1.28 \times Chl + 8.33$	15.43	0.12	-1.42

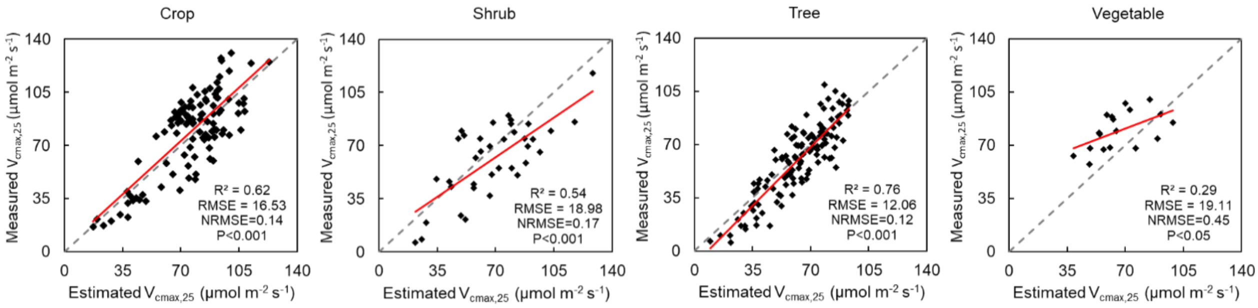

Figure 4. Relationships between estimated and measured $V_{cmax,25}$ for crops, shrubs, trees and vegetables.

9. Figure 4: Please change the axes so the observed values are on the y-axis. This makes it so the intercept term is interpretable in terms of the linear relationship between the two variables.

Response:

As shown in Figure 4, we have changed the measured $V_{cmax,25}$ to the y-axis.