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

General comments

1. It is not explained why those species/sites were chosen and how representative are they to the terrestrial biosphere.

   Response:
   First, the dataset should cover as many vegetation types as possible, so we sought in include as many data points, species and sites as were available to us, including crops, trees, shrubs and vegetables. Secondly, Spatial difference should be considered. We added data from Canada, which made our data more spatially diverse (i.e. different environmental growing conditions). Moreover, considering the convenience of the experiments, most of data was observed in and around Beijing.

   Revisions:
   2.1 Study sites and samples
   …The Borden Forest Research Station has a humid continental climate. The mean annual temperature is about 7.4°C and the mean annual precipitation is 784 mm (Froelich et al., 2015). We sought to include as many species and plant functional types as possible in the dataset, the sample species used included crops, shrubs, trees and vegetables. During field sampling we choose leaves that were representative of the plants at the site, and sampled light-adjusted, top of canopy leaves. Data were taken from 283 leaf samples, including cotton, wheat, forsythia and so on. Further details are shown in Table 1.

2. What is the measurement temperature of leaf gas exchange and how the Vcmax and Jmax are temperature corrected?

   Response:
   In China, the leaf chamber temperature was kept constant close to the air temperature during the measurement. Vcmax and Jmax are temperature normalized to 25°C using 'plantecophys', an R package. At the Borden Research Station, the leaf chamber was maintained as close to 25°C as possible. All values of Vcmax and Jmax parameters were scaled to a reference temperature of 25°C using the Arrhenius

equation. We have added some descriptions in Section 2.3.

Revisions:

2.3 Gas exchange measurements and determination of $V_{cmax,25}$ and $J_{max,25}$

…Throughout the measurement sequence, the leaf chamber temperature was kept constant close to the air temperature and the relative humidity was kept the same as the relative humidity of the air. The values of the photosynthetic parameters – $V_{cmax,25}$ and $J_{max,25}$ – were obtained from the fitted A–Ci curves using 'plantecophys', an R package for processing leaf gas exchange data (Duursma, 2015). At the Borden Research Station, A–Ci curves of the leaves were plotted for a photosynthetic photon flux density of 1800 µmol m^{-2} s^{-1}, and CO_2 concentrations of 400, 200, 100, 50, 400, 400, 600, 800, 1000, 1200, 1500 and 1800 µmol CO_2 mol^{-1} air. Throughout the measurement, the leaf chamber was maintained as close to 25°C as possible (approximately ±1°C) and relative humidity kept between 40% and 80%. $V_{cmax,25}$ and $J_{max,25}$ were calculated from the A-Ci curves fitted using a curve-fitting tool developed by Kevin Tu (www.landflux.org) following Ethier and Livingston (2004), and scaled to a reference temperature of 25°C using the Arrhenius equation (Sharkey et al., 2007)…

3. The linear regression analysis did not rule out the possibility of inter-species variation in the Vcmax-chlorophyll content relationship. And the data set is too small with very limited coverage of terrestrial ecosystems. The use of the word 'converge' is thus not conclusive. The empirical nature of this study suggests that a mechanistic understanding of the variation of photosynthetic capacity is still absent at global scales and the application of conclusions from this study should be within the species and sites tested.

Response:

In the study, the data set is indeed small with limited coverage of terrestrial ecosystems. 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. 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. Reviewer1 was concerned that our dataset is not sufficiently representative to justify the claim of a 'universal' relationship. You thought that the use of the word 'converge' is thus not conclusive. 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.

4. There is an underlying chain of assumptions in this study. That is (1) Jmax should relate to chlorophyll content convergently among C3 species; (2) Jmax/Vcmax is generally a constant; (3) Vcmax thus should relate to chlorophyll content with a relationship that does not vary among species. The authors have not demonstrated assumption (1) being a widely accepted scientific fact. But Let's assume assumption (1) is true. As the authors noted, the Jmax/Vcmax could vary from 1 to 3 which is not a small change and could completely throw off the relationship between Vcmax and chlorophyll content, preventing a universal relationship. With a limited number of species tested in this study, it is difficult to separate the importance of Jmax/Vcmax. The chain of logic is inadequately supported. The attempt of finding physiological explanation of the Vcmax and chlorophyll content relationship is thus incomplete.

Response:

The study demonstrated assumption (1) from the theoretical and experimental perspectives. Theoretically, leaf J_{max} and chlorophyll prove to have a strong consistency in section 4.2. Experimentally, Equations (9) and Equation (10) have similar slopes, suggesting a relatively robust relationship between leaf $J_{cmax,25}$ and chlorophyll content across species and regions. For assumption (2), we have read more literatures and found that the J_{max}/V_{cmax} ratio is around 2, which may support the logical chain. We have adjusted and added some descriptions in Section 4.2 and 4.3 to address the reviewer's comments.

Revisions:

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 basis to the relationship between leaf $V_{cmax,25}$ and the chlorophyll content.

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

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

（2）Relationships between J_{max} and V_{cmax}

Studies have shown that the J_{max}/V_{cmax} ratio can represent the limited difference in photosynthesis between two photosynthetic systems, namely, electron transfer and Rubisco carboxylation. Wullschleger (1993) integrated and analyzed the V_{cmax} and J_{max} values of 109 species and showed that the ratio between J_{max} and V_{cmax} was constant despite the differences in growth environment and species. This allowed V_{cmax} to be modeled using the chlorophyll-derived J_{max} value:

$$J_{max} = 1.64 \times V_{cmax}(\mu mol\ \ m^{-2}s^{-1}) + 29.1(\mu mol\ \ m^{-2}s^{-1}) \quad (11)$$

Equation (12) in this paper represents a strong relationship between $J_{max,25}$ and $V_{cmax,25}$ across different C_3 plants. In order to make a comparison with previous research results, we natural-log-transformed the $J_{max,25}$ and $V_{cmax,25}$ values in our dataset and analyzed them using a linear regression (Table 2). All four datasets were found to have similar slope parameters, ranging from 0.75 for the TRY data to 0.91 for our dataset, and R^2 values greater than 0.78.

$$J_{max,25} = 2.17 \times V_{cmax,25}(\mu mol\ \ m^{-2}s^{-1}) + 0.37(\mu mol\ \ m^{-2}s^{-1}) \qquad R^2 = 0.79 \quad (12)$$

Some studies have found that the ratio between $J_{max,25}$ and $V_{cmax,25}$ significantly decreases with increasing growth temperature (Kattge and Knorr, 2007;Wang et al., 2017). Generally, the activity of Rubisco and the photosynthetic electron transport chain are in equilibrium and coordination under normal light conditions. However, the effect of a reduction in light intensity on the photosynthetic electron transport rate is more obvious than the effect on Rubisco activity. A decrease in light intensity will result in a decrease in the J_{max}/V_{cmax} ratio. The J_{max}/V_{cmax} ratio has been found to shift, between the range of about 1 to 3 – reflecting the co-regulation of RuBP carboxylation and regeneration (Wullschleger, 1993;Leuning, 1997;Medlyn et al., 1999;Leuning, 2002;Medlyn et al., 2002). Nonetheless, the J_{max}/V_{cmax} ratio is generally found to be around 2 (Dillen et al., 2012;Rogers et al., 2017), which is also shown in Eq. (11) and Eq. (12).

5. Overall, the concept of this article is interesting and important. The presentation of the content could include more details and analyses. The discussion could consider a more comprehensive comparison of literatures. The title and conclusions could benefit from less extrapolation. I suggest the paper could be more useful to the community after addressing these limitations.

Response:

In the discussion section, we added the comparison of the relationships between leaf $V_{cmax,25}$ with both chlorophyll and nitrogen content. We also made some revisions to the title, discussion and conclusion section.

Revisions:

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

4 Discussion

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.

5 Conclusions

Thus far, most attention from the research community has been focused on using leaf nitrogen content to retrieve $V_{cmax,25}$ because nitrogen is the main component of both Rubisco and the light-harvesting complexes that regulate photosynthesis. However, deriving spatially continuous estimates of nitrogen content that correspond to Rubisco at a large spatial scale has proved complex. Leaf chlorophyll is responsible for light harvesting in photosynthesis and is theoretically closely related to $J_{max,25}$. The relationship between $V_{cmax,25}$ and $J_{max,25}$ provides more possibilities for inverting $V_{cmax,25}$, a key parameter in most terrestrial biosphere models, from chlorophyll content. In this study, the potential for using leaf chlorophyll content to retrieve leaf $V_{cmax,25}$ across different C_3 plant types was investigated. 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). 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.

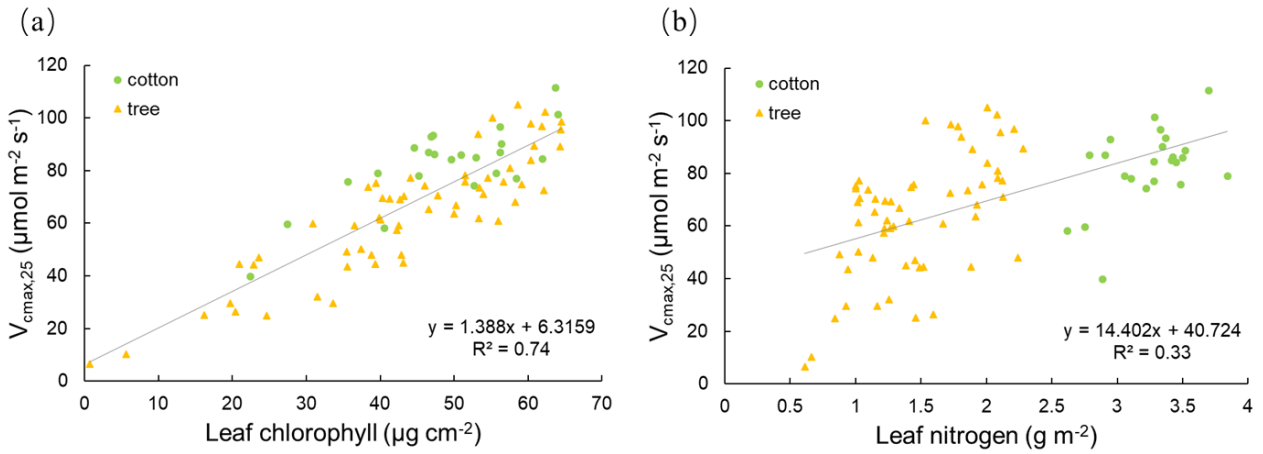

Figure 5. Relationships between leaf $V_{cmax,25}$ and (a) chlorophyll; (b) nitrogen for cotton in 2017 and trees in 2014.

Specific comments

1. Consider subscribe 'cmax' and 'max' in the terms 'Vcmax' and 'Jmax'.

   Response:
   'Vcmax', 'Vcmax$_{25}$', 'Jmax' and 'Jmax$_{25}$' have been changed to 'V_{cmax}', '$V_{cmax,25}$', 'J_{max}' and '$J_{max,25}$' throughout the manuscript, respectively.

2. L38: The authors mentioned 'most classical biochemical models' but did not provide citation. I suggest the authors to consider the work by Rogers et al. (2017).

   Response:
   We have added some relevant references to the sentence.

   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)….

3. L64 -70: The authors used the word 'chlorophyll' without defining the exact meaning. As the authors are aware, chlorophyll content, chlorophyll index, and chlorophyll activity could be very different. I suggest the authors to clarify what is use by each study.

   Response:
   We thank the reviewer for highlighting this and have modified the manuscript accordingly.

4. L96-105: There is a valuable potential for the authors to validate the equation 1 (i.e., Chl and SPAD relationship; Markwell 1995) with the spectrophotometer method (Croft 2017). However, the authors used the empirical model from Markwell without considering whether the relationship applies to all their species. The SPAD measures the chlorophyll index which needs to be calibrated to each species/site to translate to

leaf chlorophyll content. The adoption of Markwell equation needs justification.

Response:
The SPAD measures leaf transmittance at two wavelengths: red (650 nm), where there is strong absorption by chlorophyll, and near-infrared (940 nm), where there is no absorption by chlorophyll. It has been widely used in rapid, quantitative leaf chlorophyll measurements taken in the field. As shown in Figure S1, we collected the publications reporting calibration curves for the SPAD. All the curves are within a small range, which seems to indicate that it is feasible to adopt Markwell equation.

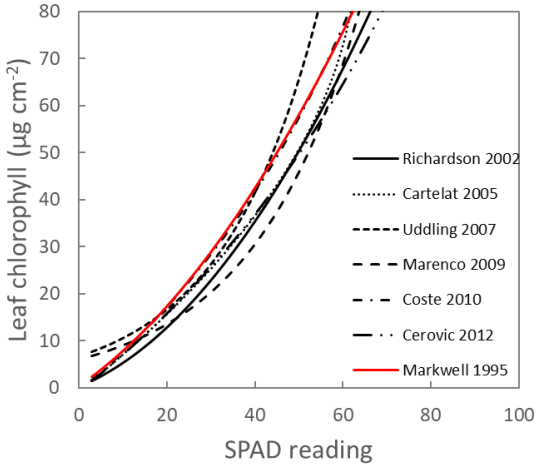

Figure S1. Comparison of the calibration models for SPAD-502 available in the literature. The functions for the eight models plotted on the graph were: $y = 0.08982(10.6+7.39x+0.114x^2)$ for soybean and maise (Markwell et al., 1995); $y = 0.552 + 0.404x + 0.0125x^2$ for paper birch (Richardson et al., 2002); $y = 93.6 – 11.9\sqrt{(62 – x)}$ for wheat (Cartelat et al., 2005); $y = 6.91\ e^{(0.0459x)}$ for birch, wheat and potato (Uddling et al., 2007); $y = 6.205\ e^{(0.0408x)}$ for six tropical tree species (Marenco et al., 2009); $y = (117.1x)/(148.84 – x)$ for thirteen tropical tree species (Coste et al., 2010); $y = (138x)/(185 – x)$ for kiwi, grapevine, wheat and maize (Cerovic et al., 2012).

[1] Markwell, J., Osterman, J. C., and Mitchell, J. L.: Calibration of the Minolta SPAD-502 leaf chlorophyll meter, Photosynthesis Res., 46, 467–472, 10.1007/BF00032301 1995.
[2] Richardson, A. D., Duigan, S. P., and Berlyn, G. P.: An Evaluation of Noninvasive Methods to Estimate Foliar Chlorophyll Content, New Phytol., 153, 185-194, 10.2307/1513920, 2002.
[3] Cartelat, A., Cerovic, Z. G., Goulas, Y., Meyer, S., Lelarge, C., Prioul, J. L., Barbottin, A., Jeuffroy, M. H., Gate, P., and Agati, G.: Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (Triticum aestivum L.) ☆, Field Crops Res., 91, 35-49, 10.1016/j.fcr.2004.05.002, 2005.
[4] Uddling, J., Gelang-Alfredsson, J., Piikki, K., and Pleijel, H.: Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings, Photosynthesis Res., 91, 37-46, 10.1007/s11120-006-9077-5, 2007.
[5] Marenco, R. A., Antezana-Vera, S. A., and Nascimento, H. C. S.: Relationship between specific leaf area, leaf thickness, leaf water content and SPAD-502 readings in six Amazonian tree species, Photosynthetica, 47, 184-190, 10.1007/s11099-009-0031-6, 2009.
[6] Coste, S., Baraloto, C., Leroy, C., Marcon, É., Renaud, A., Richardson, A. D., Roggy, J. C., Schimann, H., Uddling, J., and Hérault, B.: Assessing foliar chlorophyll contents with the SPAD-502 chlorophyll

meter: a calibration test with thirteen tree species of tropical rainforest in French Guiana, Annals of Forest Science, 67, 607, 10.1051/forest/2010020, 2010.
[7] Cerovic, Z. G., Masdoumier, G., Ghozlen, N. B., and Latouche, G.: A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids, Physiol. Plant., 146, 251-260, 10.1111/j.1399-3054.2012.01639.x, 2012.

5. One tree species (white ash) presents in both Ontario and Beijing. Could the authors show how the chlorophyll content from two sites compare?

Response:
We compared the chlorophyll content of white ash in Beijing and Ontario in Figure S2. The measurement date of white ash in Beijing were October 12 and 13, 2017. The samples were chosen to represent as wide a range of leaf chlorophyll content as possible, with colours ranging from yellowish green to dark green. The measurement date of white ash in Ontario were covering the whole growing seasons in 2014 and 2015. Therefore, the chlorophyll content exhibits a seasonal variation. The chlorophyll content range of the white ash in two areas is generally the same.

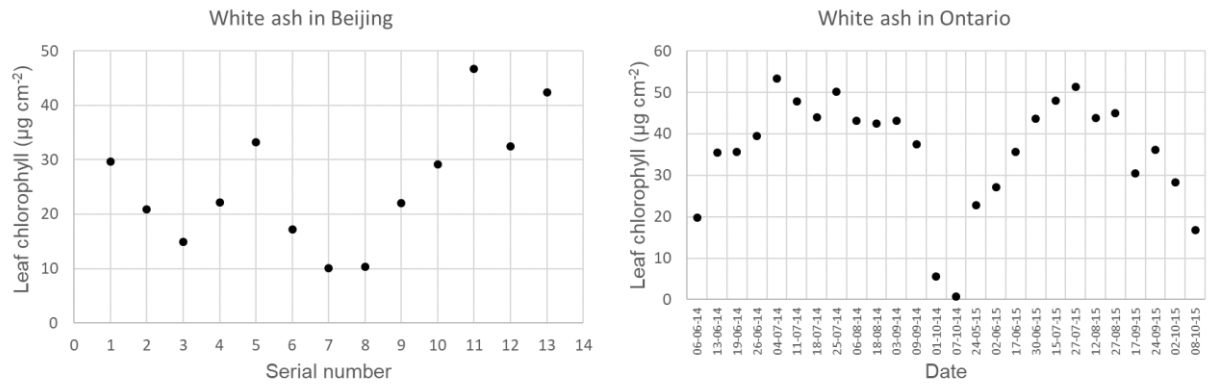

Figure S2. Leaf chlorophyll content of white ash in Beijing and Ontario.

6. L110: the conditions (leaf temperature, VPD, PAR) in the licor chamber as well as the outside air are important but missing. The soil moisture condition could also affect photosynthetic capacity (e.g., Keenan et al., 2010). I suggest the authors to also exclude the impact of soil moisture.

Response:
We have added the descriptions about the conditions in the leaf chamber in Section 2.3. The plant we measured were all under normal physiological conditions and were not stressed by various environmental factors, such as nutrient deficiencies, drought and heat, pests and diseases. We added a line to clarify this point in the paper.

Revisions:
2.3 Gas exchange measurements and determination of $V_{cmax,25}$ and $J_{max,25}$
…All leaves were measured under environmental conditions that were within the plants normal range, and were not visibly under stress. In addition to meteorological conditions, soil moisture has for instance also

been found to affect V_{cmax} (Keenan et al., 2010, Chen et al., 2019), but no soil moisture limitation was present at the sites.

7. L126-129: The content seems to belong to discussion more than result.

Response:
We have deleted this content because it is also not suitable for the discussion section, and doesn't add much to the paper.

8. Fig 2: The figure did not convincingly show chlorophyll content as a good predictor of Vcmax. The general patterns of the two variables are similar but that could be simple plant phenology. What is more important is the short-term variation of Vcmax, which chlorophyll content completely missed. This figure suggests to me that chlorophyll content is nothing but a proxy of leaf phenology, which one can derive from satellite vegetation index. More convincing evidence showing chlorophyll content could capture Vcmax variation is needed.

Response:
In the short term, leaf chlorophyll content should be stable, and leaf Vcmax will change slightly as the environment changes. Even if the values of Vcmax are normalized to 25°C, the effects of other environmental factors on Vcmax cannot be completely eliminated. The cotton leaves with different biochemical parameters were measured from July 7 to July 10, 2017, totally four days. As shown in Figure S3, leaf chlorophyll content has a good relationship with Vcmax for data collected within four days.

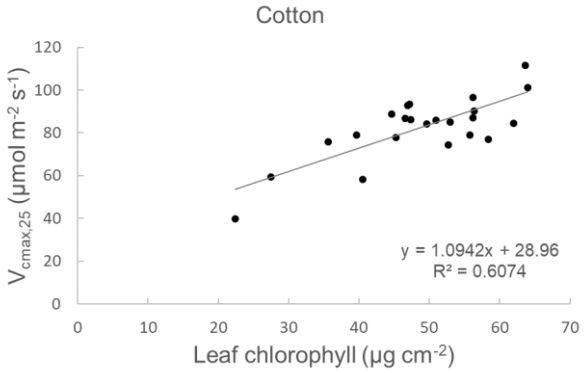

Figure S3. Relationship between leaf $V_{cmax,25}$ and chlorophyll content for cottons from July 7 to July 10, 2017.

9. L159-175: Initially, I got very confused by the information in this section. Later after reading the whole of discussion, it occurs to me this section is roughly an overview of theories to support the following discussions. I suggest the authors to reorganise this section, so that the connection of mentioning all those studies and to the rest of discussion is clear.

Response:
Thank you for the suggestion. In order to make the section clear, we have adjusted the logic of this section to correspond to Approach 2 in Section 4.3.

Revisions:

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 believe that there is a relationship between leaf $V_{cmax,25}$ and the chlorophyll content.

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

…

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

（1） Relationships between J_{max} and chlorophyll

It has been shown that the number of photons harvested by a leaf is related to chlorophyll content across lots of different plant species (Evans, 1996;Evans and Poorter, 2001). The potential rate of electron transport J (μmol m^{-2} s^{-1}) depends on the leaf-absorbed PAR (Φ; μmol m^{-2} s^{-1}) according to the following equation:

$$0.7\times J^2-(J_{max}+0.385\times\Phi)\times J+0.385\times J_{max}\times\Phi=0 \quad (8)$$

Therefore, the electron transport rate is a function of incident PAR and the efficiency of light-harvesting apparatus, which includes chlorophyll (Croft et al., 2017). Measurements on barley by Nolan and Smillie (1976), both douglas fir and poplar by Ripullone et al. (2003), forbs by Singsaas et al. (2004), and sweetgum by Warren et al. (2015) suggest a linear correlation between $J_{max,25}$ and leaf chlorophyll content (Alton, 2017):

$$J_{max,25}=2.40\times Chl(\mu g\ cm^{-2})+24(\mu mol\ m^{-2}s^{-1}) \quad (9)$$

In this study, a relationship between leaf $J_{max,25}$ and chlorophyll was established. Comparing Eq. (10) with Eq. (9), the two leaf $J_{max,25}$–Chl models can be seen to have similar slopes, suggesting a relatively robust correlation between leaf $J_{cmax,25}$ and chlorophyll content across species and regions:

$$J_{max,25}=2.78\times Chl(\mu g\ cm^{-2})+18.45(\mu mol\ m^{-2}s^{-1}) \qquad R^2=0.51 \quad (10)$$

（2）Relationships between J_{max} and V_{cmax}

Studies have shown that the J_{max}/V_{cmax} ratio can represent the limited difference in photosynthesis between two photosynthetic systems, namely, electron transfer and Rubisco carboxylation. Wullschleger (1993) integrated and analyzed the V_{cmax} and J_{max} values of 109 species and showed that the ratio between J_{max} and V_{cmax} was constant despite the differences in growth environment and species. This allowed V_{cmax} to be modeled using the chlorophyll-derived J_{max} value:

$$J_{max} = 1.64 \times V_{cmax}(\mu mol\ m^{-2}s^{-1}) + 29.1(\mu mol\ m^{-2}s^{-1}) \quad (11)$$

Equation (12) in this paper represents a strong relationship between $J_{max,25}$ and $V_{cmax,25}$ across different C_3 plants. In order to make a comparison with previous research results, we natural-log-transformed the $J_{max,25}$ and $V_{cmax,25}$ values in our dataset and analyzed them using a linear regression (Table 2). All four datasets were found to have similar slope parameters, ranging from 0.75 for the TRY data to 0.91 for our dataset, and R^2 values greater than 0.78.

$$J_{max,25} = 2.17 \times V_{cmax,25}(\mu mol\ m^{-2}s^{-1}) + 0.37(\mu mol\ m^{-2}s^{-1}) \quad R^2 = 0.79 \quad (12)$$

Some studies have found that the ratio between $J_{max,25}$ and $V_{cmax,25}$ significantly decreases with increasing growth temperature (Kattge and Knorr, 2007;Wang et al., 2017). Generally, the activity of Rubisco and the photosynthetic electron transport chain are in equilibrium and coordination under normal light conditions. However, the effect of a reduction in light intensity on the photosynthetic electron transport rate is more obvious than the effect on Rubisco activity. A decrease in light intensity will result in a decrease in the J_{max}/V_{cmax} ratio. The J_{max}/V_{cmax} ratio is said to be changing – within the range of about 1to 3 – reflecting the co-regulation of RuBP carboxylation and regeneration (Wullschleger, 1993;Leuning, 1997;Medlyn et al., 1999;Leuning, 2002;Medlyn et al., 2002). However, some researches have shown that the J_{max}/V_{cmax} ratio is around 2 (Dillen et al., 2012;Rogers et al., 2017), which is also shown in Eq. (11) and Eq. (12).

（3）Relationships between V_{cmax} and chlorophyll

Combinations of the equations relating J_{max} to leaf chlorophyll and J_{max} to V_{cmax} were used to construct the models relating V_{cmax} to leaf chlorophyll presented in Table 3. 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}. All of the models produced only a slight overestimation or underestimation, with biases of -4.64, -6.78, 3.05 and -1.50 μmol m^{-2} s^{-1}, as shown. The results indicate that there is a stable relationship between leaf V_{cmax} and chlorophyll content, and the physiological basis of the stable relationship relating J_{max} to leaf chlorophyll and J_{max} to V_{cmax} is also confirmed.

The fact that the J_{max}/V_{cmax} relationship applies well across different plant types may strengthen the theoretical basis for using chlorophyll to estimate $V_{cmax,25}$ for different C_3 plant types. First, it is difficult to accurately obtain leaf nitrogen fractions based on Rubisco from remote sensing data, which leads to the use of a proxy, namely leaf chlorophyll. Moreover, establishing a direct stable relationship between leaf chlorophyll and V_{cmax} for different plants not only avoids the errors caused by the unstable relationship between leaf chlorophyll and nitrogen, but also eliminates the uncertainties inherent in the classification of plant types. More importantly, some operational instruments, such as the MERIS and Sentinel-3 OLCI, can collect global multi-spectral remote sensing data in the red-edge band. 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.

10. L236-238 and fig 3: A stable linear relationship between Vcmax and chlorophyll content across species

does not necessarily means the variation among species does not have significant impacts of the slope. The plots of each species suggest to me that the model is biased but the bias (slope and interceptor) is slightly different in each species. The authors could consider a linear mixed effect model with species as random factor to rule out the impact of species.

Response:

Thank you for the suggestion. We built a linear mixed effect model with chlorophyll as fixed effect and species as random effect. In random intercept model, only the intercepts are different between species, the slopes are the same. As shown in Table S1, the variance of chlorophyll is 218.992 and the variance of species is 0.022. This shows that chlorophyll causes a greater proportion of variation. In random slope model, not only the intercepts are different between species, but also the slopes are different. As shown in Table S2, the variance of chlorophyll is 209.968, and the intercept and slope variances of species are 125.891 and 0.030, respectively. Moreover, the difference between intercepts of species is not statistically significant (P = 0.449), and the difference between the slopes is also not statistically significant (P = 0.484). Therefore, the result shows that the species have no significant impacts on the values of $V_{cmax,25}$.

Table S1. Estimates of Covariance Parameters[a] in random intercept model.

Parameter		Estimate	Std. Error	Wald Z	Sig.
Residual		218.992	18.587	11.782	0.000
Chlorophyll [subject = species]	Variance	0.022	0.022	1.043	0.297

a.Dependent Variable: $V_{cmax,25}$.

Table S2. Estimates of Covariance Parameters[a] in random slope model.

Parameter		Estimate	Std. Error	Wald Z	Sig.
Residual		209.968	18.180	11.550	0.000
Intercept [subject = species]	Variance	125.891	166.336	0.757	0.449
Chlorophyll [subject = species]	Variance	0.030	0.044	0.700	0.484

a.Dependent Variable: $V_{cmax,25}$.

Revisions:

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

… Moreover, a linear mixed effect model with species as random factor was conducted to rule out the impact of species. The results show that the species has no significant impacts on the values of $V_{cmax,25}$ ($P > 0.05$)…

11. L257: The promise of the conclusion in this study could be readily used worldwide to estimate Vcmax is misleading. I agree this study is one step toward such an application, but a lot more data still needed before a generalisable relationship could be determined.

Response:

We have revised the relevant expression in the conclusion section.

Revisions:

5 Conclusions

Thus far, most attention from the research community has been focused on using leaf nitrogen content to retrieve $V_{cmax,25}$ because nitrogen is the main component of both Rubisco and the light-harvesting complexes that regulate photosynthesis. However, deriving spatially continuous estimates of nitrogen content that correspond to Rubisco at a large spatial scale has proved complex. Leaf chlorophyll is responsible for light harvesting in photosynthesis and is theoretically closely related to $J_{max,25}$. The relationship between $V_{cmax,25}$ and $J_{max,25}$ provides more possibilities for inverting $V_{cmax,25}$, a key parameter in most terrestrial biosphere models, from chlorophyll content. In this study, the potential for using leaf chlorophyll content to retrieve leaf $V_{cmax,25}$ across different C_3 plant types was investigated. 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 19.11 $\mu mol\ m^{-2}\ s^{-1}$ for crops, shrubs, trees and vegetables, respectively). 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.