C₃ plants converge on a universal relationship between leaf maximum carboxylation rate and chlorophyll content

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Abstract. The leaf maximum carboxylation rate (Vcmax) is one of the crucial parameters in determining the photosynthetic capacity of plants. Providing accurate estimates of leaf $Vcmax_{25}$ that cover large geographic areas and that incorporate plant seasonality is central to correctly predicting carbon fluxes within the terrestrial global carbon cycle. Chlorophyll, as the main photon-harvesting component in leaves, is closely linked to photosynthesis. However, how the nature of the relationship between the leaf maximum carboxylation rate scaled to 25°C ($Vcmax_{25}$) and leaf chlorophyll content varies according to plant type is uncertain. In this study, we investigate whether a universal and stable relationship exists between leaf $Vcmax_{25}$ and leaf chlorophyll content across different C₃ plant types from a plant physiological perspective and verify it using field experiments. Measurements of leaf chlorophyll content (Chl) and CO_2 response curves were made on 283 crop, shrub, tree and vegetable leaves in China and the Borden Forest Research Station in southern Ontario, Canada. A strong relationship was found between the leaf $Vcmax_{25}$ and chlorophyll content across different C₃ plant types ($R^2 = 0.65$, P<0.001). Validation showed that the model performs well, producing relatively low normalized root mean square errors (NRMSE) for crops (0.14), shrubs (0.17), trees (0.12) and vegetables (0.45). The results confirm that leaf chlorophyll content can be a reliable proxy for estimating $Vcmax_{25}$ across different C₃ plant types over space and time, opening the door to accurate spatially continuous estimates of $Vcmax_{25}$ at the global scale.

1 Introduction

Photosynthesis is a core component of the land ecosystem carbon cycle, serving as the material basis and energy source for life on Earth (Sellers et al., 1997;Canadell et al., 2007). Accurately simulating photosynthesis is a defining prerequisite for predicting vegetation productivity and the carbon cycle, for quantifying global carbon budgets, identifying the spatial−temporal distribution of carbon sources and sinks, and predicting changes in plant ecophysiology in response to a changing climate. Annually, plant photosynthetic flux amounts to an estimated 128 PgC year[-1] (Beer et al., 2010), forming an important part of the terrestrial carbon sink, which is estimated to have removed 3.61 PgC year[-1] from the atmosphere during

the period 2007–2016, amounting to approximately one-fifth of the atmospheric carbon content every year (Keenan and Williams, 2018).

Currently, most terrestrial biosphere models simulate photosynthesis using a leaf biochemical model developed by Farquhar et al. (1980). The leaf photosynthetic capacity is usually characterized by two key parameters: the leaf maximum
35   carboxylation rate ($Vcmax_{25}$) and the leaf maximum electron transport rate ($Jmax_{25}$) scaled to 25°C (Farquhar et al., 1980). $Jmax_{25}$ is related to the leaf's ability to transport electrons to produce adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), which are then used to drive the carbon reactions. $Vcmax_{25}$ determines the leaf's capacity to fix carbon into sugars within the reactions of the Calvin−Benson cycle. In most classical biochemical models, $Vcmax_{25}$ is usually hypothesized to be a fixed value for a given plant functional type. However, even within the same plant
40   type, $Vcmax_{25}$ can vary observably with space and time, resulting in an inaccurate estimation of the photosynthesis (Kattge et al., 2009;Croft et al., 2017).

The traditional approach to measuring leaf $Vcmax_{25}$ using gas exchange experiments is time-consuming and resource-heavy. There are also several uncertainties inherent in the measurement of $Vcmax_{25}$ and $Jmax_{25}$ (Ali et al., 2015). First, when estimating Rubisco abundance from CO_2 response curves (A−Ci curves), it is assumed that Rubisco is fully activated.
45   However, some studies have shown that Rubisco usually operates in a manner that is below maximum activity or exceeds CO_2 fixation requirements (Stitt and Schulze, 1994;Warren et al., 2000). Secondly, methods used to fit the Farquhar model are not yet consistent (Manter and Kerrigan, 2004;Dubois et al., 2007;Miao et al., 2009). Thirdly, it is often challenging to obtain accurate or biologically realistic estimates of dark respiration (Medlyn et al., 2002b). Therefore, it is highly desirable to explore a general and reliable approach to large-scale estimation of $Vcmax_{25}$.

50   As a key parameter in photosynthesis, $Vcmax_{25}$ is inevitably related to plant functional traits (Serbin et al., 2012;Croft et al., 2017;Smith et al., 2019). Since Rubisco is rich in nitrogen, some studies have illustrated that Rubisco has a linear relationship with leaf nitrogen content per unit area for certain plant species and leaf growth stages (Medlyn et al., 1999;Kattge et al., 2009). However, there are still challenges involved in retrieving $Vcmax_{25}$ through its correlation with the leaf nitrogen content. First, total leaf nitrogen content is not necessarily an accurate proxy for $Vcmax_{25}$ because of the large
55   and dynamic partitioning of leaf nitrogen between the photosynthetic and non-photosynthetic components, and between Rubisco and light-harvesting fractions (Kenzo et al., 2006;Croft et al., 2017;Luo et al., 2018). In this regard, it is the nitrogen fraction that corresponds to the Rubisco that is expected to show a strong relationship with $Vcmax_{25}$ (Niinemets and Tenhunen, 1997). 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
60   leaf nitrogen content from satellite observation data. In this case, an alternative approach to estimating $Vcmax_{25}$ 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).

Some researchers have explored the relationships between leaf $Vcmax_{25}$ and chlorophyll content or indicators associated with chlorophyll (Qian et al., 2019). Croft et al. (2017) carried out field measurements on leaf biochemistry and gas

65 exchange in a deciduous forest, and the results indicated that chlorophyll provides an ideal proxy for leaf $Vcmax_{25}$. Luo et al. (2018) used chlorophyll to constrain $Vcmax_{25}$ in a terrestrial biosphere model to significantly reduce the uncertainties in estimates of gross primary productivity and evapotranspiration. Houborg et al. (2013) summarized a semi-mechanistic approach for coupling leaf $Vcmax_{25}$ with chlorophyll based on relationships between $Vmax_{25}$, Rubisco enzyme kinetics, leaf nitrogen, and leaf chlorophyll reported in the previous experiment results. The results indicated that chlorophyll could be

70 used as an indicator of the $Vcmax_{25}$ value. This semi-mechanistic correlation between $Vcmax_{25}$ and chlorophyll was also parameterized for a wide range of agricultural crops (Houborg et al., 2015). Alton (2017) developed a novel retrieval method for canopy-scale $Vcmax_{25}$ based on a linear correlation between leaf $Jmax_{25}$ and chlorophyll content and a quasi-linear relationship between $Jmax_{25}$ and $Vcmax_{25}$. Dillen et al. (2012) found that the Photochemical Reflectance Index, Chlorophyll Normalized Difference Index and, in particular, the red-edge position, which is sensitive to chlorophyll content, had the

75 potential be good indicators of $Vcmax_{25}$. Measurements by Qian et al. (2019) demonstrated a reliable relationship between leaf $Vcmax_{25}$ and chlorophyll content; all the vegetation indices that are sensitive to chlorophyll were significantly related to $Vcmax_{25}$.

The above studies illustrated a significant relationship between leaf $Vcmax_{25}$ and chlorophyll content for many plant species. However, it remains unknown whether the relationship between leaf $Vcmax_{25}$ and chlorophyll content across

80 different C_3 plant types is uniform. This study attempts to explore the relationship between leaf $Vcmax_{25}$ and chlorophyll content from a plant physiological perspective and verify it using field experiments. This should help to provide an operational approach for the global mapping of $Vcmax_{25}$ across different plant types.

2 Materials and methods

2.1 Study sites and samples

85 Field experiments took place in four areas, including Baoding, Hebei province (38°10′–40°00′ N, 113°40′–116°20′ E), Beijing (39°24′–41°36′ N, 115°42′–117°24′ E), Sanya, Hainan province (18°09′–18°37′ N, 108°56′–109°48′ E), and the Borden Forest Research Station, located in southern Ontario, Canada (44°19′ N, 79°56′ W) (Froelich et al., 2015). Baoding has a temperate continental monsoon climate. The mean annual temperature is approximately 13.4°C and the mean annual precipitation about 498.9 mm. Beijing has the same climate type as Baoding, with a mean annual temperature of

90 approximately 12.6°C and mean annual total precipitation of about 620.6 mm. Sanya has a tropical maritime monsoon climate, with a mean annual temperature of 21.4°C and mean annual precipitation of 1347.5 mm. 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). The sample species used included crops, shrubs, trees and vegetables. Data were taken from 283 leaf samples, including cotton, wheat, forsythia and so on. Further details are shown in Table 1.

2.2 Estimation of leaf chlorophyll content

Two methods were used to estimate the leaf chlorophyll content. For the Chinese samples, a portable SPAD-502 chlorophyll meter (Konica Minolta Inc., Osaka, Japan) was used to non-destructively measure leaf chlorophyll. The SPAD-502 meter gives a unitless measurement value, claiming an accuracy of ± 1 SPAD unit (Houborg et al., 2013). Five separate measurements were made on each leaf using a SPAD meter to determine the leaf chlorophyll content. In order to convert the SPAD readings (unitless) into absolute values of chlorophyll content, an empirical statistical model developed by Markwell et al. (1995) was used:

$$Chl(\mu mol\ m^{-2}) = 0.114 \times (SPAD)^2 + 7.39 \times (SPAD) + 10.6 \qquad R^2 = 0.96 \qquad (1)$$

At the Borden Research Station, the leaf samples were sealed under cool conditions to keep the leaves fresh and prevent moisture loss. The leaves were then taken back to the laboratory for subsequent biochemical analysis. Foliar chlorophyll was extracted using spectra-analysed grade *N, N*-dimethylformamide, and the absorbance was measured using a Shimadzu UV-1700 spectrophotometer (Wellburn, 1994;Croft et al., 2017).

2.3. Gas exchange measurements and determination of $Vcmax_{25}$ and $Jmax_{25}$

At all sites, the LI-6400 portable infrared gas analyzer (LI-COR, Lincoln, NE, USA), which has an artificial red/blue light source, was used for gas exchange measurements. In China, A−Ci curves were produced for stepwise ambient CO_2 concentrations of 400, 300, 200, 100, 50, 400, 400, 600, 900, 1200 and 1500 μmol CO_2 mol^{-1} air. Each complete step in the A−Ci curves took at least 60 seconds and up to 200 seconds to complete. 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. The values of the photosynthetic parameters − $Vcmax_{25}$ and $Jmax_{25}$ − were obtained from the fitted A−Ci curves using 'plantecophys', an R package for processing leaf gas exchange data (Duursma, 2015).

3 Results

3.1 Distribution of chlorophyll content and $Vcmax_{25}$

Figure 1 illustrates the distribution of leaf chlorophyll content and $Vcmax_{25}$ values. The minimum and maximum values of chlorophyll content found in the samples were 0.7 and 91.6 μg cm^{-2}, respectively. Samples with chlorophyll content ranging from 50−60 μg cm^{-2} accounted for the largest proportion of values. $Vcmax_{25}$ values varied between 5.5 and 130.8 μmol m^{-2} s^{-1}, with values in the 60−100 μmol m^{-2} s^{-1} range being the most abundant, which is consistent with reported values for C_3 plants in the classical biochemical model (Kattge et al., 2009). Even for the same plant type, $Vcmax_{25}$ values differed by up to a factor of 20, indicating the large variability that can exist (Fig. 1(b)). It is noteworthy that, for the four plant types, the

variations in $Vcmax_{25}$ were broadly consistent with the variations in chlorophyll content. Crops and vegetables both displayed markedly higher $Vcmax_{25}$ values than shrubs and trees. There was little difference in the mean leaf chlorophyll content between vegetables and trees, whereas the mean $Vcmax_{25}$ value for vegetables was roughly a third higher than that for trees. This is because plants allocate more biomass and nitrogen to the cell wall to increase leaf toughness in order to adapt to changes in the environment whereas the relative proportion of nitrogen allocated to the photosynthetic organs is reduced, resulting in a decrease in the photosynthetic capacity of the plant leaves (Hikosaka et al., 1998;Onoda et al., 2004;Warren and Adams, 2004).

3.2 Seasonal variations in leaf $Vcmax_{25}$ and chlorophyll content

As shown in Fig. 2, wheat and the four species of trees studied showed considerable temporal variation in both leaf $Vcmax_{25}$ and chlorophyll content during the growing season. Analyzing the characteristics of these seasonal variations is crucial to investigating the relationship between leaf $Vcmax_{25}$ and chlorophyll content. Since the wheat plants were measured during the period of regrowth after winter, the $Vcmax_{25}$ values were highest at the beginning. The seasonal trends in chlorophyll content were similar to those of $Vcmax_{25}$. For these tree species, the leaf $Vcmax_{25}$ values also showed seasonal variations similar to those for the chlorophyll content, increasing at the beginning of the growing season, stabilizing in the middle, and finally declining during leaf senescence. Moreover, an extremely significant linear correlation between leaf $Vcmax_{25}$ and chlorophyll content during the growing seasons was found for wheat (r = 0.84**) and for the four tree species (r = 0.80**, 0.81**, 0.89** and 0.91**).

3.3 Relationships between leaf $Vcmax_{25}$ and chlorophyll content

As shown in Fig. 3(a), the chlorophyll content was divided into 10 levels with an interval of 10 μg cm^{-2} without regard to plant type. Although leaf $Vcmax_{25}$ within each chlorophyll content level varied to a greater or lesser extent, the mean value of $Vcmax_{25}$ increased as the chlorophyll content increased. Figure 3(b) shows that the different plant types, which have different ranges of leaf $Vcmax_{25}$ and chlorophyll content, have the same slope, implying that a universal model can be applied to different C_3 plant types, regardless of differences in leaf biochemistry, structure and photosynthetic rate. Therefore, the following equation can be used to estimate $Vcmax_{25}$ from leaf chlorophyll content across different species and locations:

$$Vcmax_{25} = 1.31 \times Chl(\mu g\,cm^{-2}) + 8.52(\mu mol\,m^{-2}s^{-1}) \tag{2}$$

To test the empirical statistical model (Eq. (2)), linear regression was used to estimate leaf $Vcmax_{25}$ from leaf chlorophyll content for each plant type. Figure 4 shows that this produced a significant linear relationship between estimated and measured $Vcmax_{25}$ for crops (P < 0.001), shrubs (P < 0.001), trees (P < 0.001) and vegetables (P < 0.05). The validation of this model indicates that about 63% of the variance in the estimated $Vcmax_{25}$ can be explained by the linear regression model for crops, shrubs and trees (R^2 = 0.62, 0.54 and 0.74, respectively). The model performs well, giving relatively low normalized root mean square errors (NRMSE = 0.14, 0.17, 0.12 and 0.45 for crops, shrubs, trees and vegetables,

respectively). The accuracy of the estimated $Vcmax_{25}$ when compared to measured $Vcmax_{25}$ demonstrates that leaf chlorophyll content can be regarded as a reliable proxy for estimating $Vcmax_{25}$ across different C_3 plant types over space and

155   time.

4 Discussion

4.1 Physiological basis for the relationships between leaf $Vcmax_{25}$ and chlorophyll content

The process of photosynthesis begins with the absorption of photons mainly by chlorophyll molecules located in Photosystem II. Then specific redox reactions is initiated to supply electrons via the cytochrome b_6f complex to Photosystem

160   I, thereby producing NADPH (Porcar-Castell et al., 2014;Croft et al., 2017;Luo et al., 2018). In principle, it takes around two electrons to consume one unit of Rubisco on average, which implies a constant ratio between $Jmax_{25}$ and $Vcmax_{25}$ (Luo et al., 2018). A quasi-linear relationship is measured between $Jmax_{25}$ and $Vcmax_{25}$ (Wullschleger, 1993;Meir et al., 2002;Kattge et al., 2009;Walker et al., 2014). Vmax and Jmax have also been shown to be tightly coupled, and the ratio of $Jmax_{25}$ to $Vmax_{25}$ is typically assumed to have a fixed value in terrestrial biosphere models (Wohlfahrt et al., 1999;Leuning,

165   2002;Medlyn et al., 2002a;Kattge and Knorr, 2007).

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

170   radiation (PAR) and the efficiency of the light-harvesting complex. Theoretically, Jmax 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 $Jmax_{25}$ and chlorophyll content (Nolan and Smillie, 1976;Ripullone et al., 2003;Warren et al., 2015;Alton, 2017). Based on the above theories and assumptions, it is reasonable to believe that there is a relationship between leaf $Vcmax_{25}$ and the chlorophyll

175   content. Our observations confirm a stable chlorophyll−$Vcmax_{25}$ relationship across different C_3 plants.

4.2 Chlorophyll−$Vcmax_{25}$ relationships

Previous studies have established relationships between leaf $Vcmax_{25}$ and chlorophyll content in the following semi-mechanistic and empirical approaches. We review the different approaches that have been used to model Vcmax below, and test their accuracy in C_3 plants.

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

Houborg et al. (2013) summarized a semi-mechanistic approach for coupling leaf $Vcmax_{25}$ with chlorophyll. In accordance with Friend (1995), $Vcmax_{25}$ can be described as a function of leaf nitrogen content (N), fraction of leaf nitrogen in Rubisco (FLNR) and catalytic (Rubisco) turnover rate at 25°C ($Kcat_{25}$):

$$Vcmax_{25}\left(\frac{\mu mol_{co_2}}{m^2s}\right) = Kcat_{25}\left(\frac{mol_{co_2}}{mol_{sites}s}\right) \times \frac{8}{550}\left(\frac{mol_{sites}}{kg_{Rubisco}}\right) \times F_{LNR} \times N\left(\frac{mol_N}{m^2}\right) \times m_n\left(\frac{kg_N}{mol_N}\right) \times 6.25\left(\frac{kg_{Rubisco}}{kg_N}\right) \times 10^6 \quad (3)$$

185 where m_n is the molar mass of nitrogen (0.0140067 kg mol^{-1}). The fixed value of 6.25 ($kg_{Rubisco}$ kg_N^{-1}) converts nitrogen content to Rubisco protein content, and the constant 8/550 converts kg of Rubisco to moles of reaction sites on Rubisco molecules, assuming 8 reaction sites per molecule and a molecular mass for Rubisco of 550 kg mol^{-1} (Farquhar et al., 1980). According to Houborg et al. (2013), the C$_3$-specific value for $Kcat_{25}$ is set to 2.27 s^{-1}. According to Friend (1991), multiplying these constant terms results in the following expression:

$$Vcmax_{25}\left(\frac{\mu mol_{co_2}}{m^2s}\right) = c_0\left(\frac{mol_{sites}}{mol_N}\right) \times Kcat_{25}\left(\frac{mol_{co_2}}{mol_{sites}s}\right) \times F_{LNR} \times N\left(\frac{mol_N}{m^2}\right) \times 10^6 \quad (4)$$

190 where $c_0 = \frac{8}{550} \times 0.0140067 \times 6.25 = \frac{0.700335}{550} \approx \frac{7}{5500}$. According to the linear regression relationships between Rubisco and N, and between N and Chl for C$_3$ plant species given in Houborg et al. (2015)

$$F_{LNR} = \left(a_1 + \frac{b_1}{N}\right) \times \frac{0.16}{14.0067} \times 10^3 \quad (5)$$

and

$$N = a_2 \times Chl + b_2. \quad (6)$$

Equation (7) may be used to calculate $Vmax_{25}$ from estimates of chlorophyll content:

$$Vcmax_{25} = 1.99 \times Chl(\mu g\,cm^{-2}) - 11.52(\mu mol\,m^{-2}s^{-1}) \quad (7)$$

We tested the above approach using different plant types. Except for the vegetable samples, the estimated values were
195 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 NRMSE values (0.20, 0.28, 0.17 and 0.48 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 $Vcmax_{25}$ and chlorophyll content across different C$_3$ plants may introduce some bias.

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Approach 2: **Direct relationships between Vcmax, Jmax and chlorophyll**

（1）**Relationships between Jmax 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:

$$0.7 \times J^2 - (Jmax + 0.385 \times \Phi) \times J + 0.385 \times Jmax \times \Phi = 0 \tag{8}$$

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 Jmax$_{25}$ and leaf chlorophyll content (Alton, 2017):

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

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

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

（2）Relationships between Jmax and Vcmax

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

$$Jmax = 1.64 \times Vcmax(\mu mol\, m^{-2} s^{-1}) + 29.1(\mu mol\, m^{-2} s^{-1}) \tag{11}$$

However, some studies have found that the ratio between Jmax$_{25}$ and Vcmax$_{25}$ significantly decreases with increasing growth temperature (Kattge and Knorr, 2007;Wang et al., 2017). Fortunately, the change in the Jmax/Vcmax ratio is relatively small − within 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., 2002a). 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 Jmax/Vcmax ratio.

Equation (12) in this paper represents a strong relationship between Jmax$_{25}$ and Vcmax$_{25}$ across different C$_3$ plants. In order to make a comparison with previous research results, we natural-log-transformed the Jmax$_{25}$ and Vcmax$_{25}$ values in our

dataset and analyzed them using a linear regression (Table 2). All four datasets were found to have similar slope parameters,

230   ranging from 0.75 for the TRY data to 0.91 for our dataset, and R^2 values greater than 0.78.

$$Jmax_{25} = 2.17 \times Vcmax_{25}(\mu mol\, m^{-2}s^{-1}) + 0.37(\mu mol\, m^{-2}s^{-1}) \qquad R^2 = 0.79 \qquad (12)$$

（3）**Relationships between Vcmax and chlorophyll**

Combinations of the equations relating Jmax to leaf chlorophyll and Jmax to Vcmax were used to construct the models

relating Vcmax to leaf chlorophyll presented in Table 3. All four models were found to have similar slope parameters

235   ranging from 1.11 to 1.70, with NRMSE values < 0.15. All of the models produced only a slight overestimation or

underestimation, with biases of -4.64, -6.78, 3.05 and -1.50 $\mu mol\, m^{-2}\, s^{-1}$, as shown. The results indicate that there is a stable

relationship between leaf Vcmax and chlorophyll content, and the physiological basis of the stable relationship relating Jmax

to leaf chlorophyll and Jmax to Vcmax is also confirmed.

The fact that the Jmax/Vcmax relationship applies well across different plant types may strengthen the theoretical basis for

240   using chlorophyll to estimate $Vcmax_{25}$ 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 Vcmax 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,

245   can collect global multi-spectral remote sensing data in the red-edge band. Therefore, it may be possible to map $Vcmax_{25}$ at a

global scale using satellite remote sensing data based on the relationship between leaf $Vcmax_{25}$ and chlorophyll content.

5 Conclusions

Thus far, most attention has been focused on using leaf nitrogen content to retrieve $Vcmax_{25}$ because nitrogen is the main

component of both Rubisco and the light-harvesting complexes that regulate photosynthesis. However, deriving spatially

250   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 $Jmax_{25}$. The

relationship between $Vcmax_{25}$ and $Jmax_{25}$ provides more possibilities for inverting $Vcmax_{25}$ from chlorophyll. In this study,

the potential for using leaf chlorophyll content to retrieve leaf $Vcmax_{25}$ across different C_3 plant types was investigated. First,

for crops and forests, the seasonal variations in leaf $Vcmax_{25}$ and in chlorophyll content were found to be similar. Then, a

255   linear empirical model was built to retrieve leaf $Vcmax_{25}$ from chlorophyll content for different plant types over larger areas.

Finally, the validation results that were obtained showed that a significant linear correlation exists between estimated and

measured $Vcmax_{25}$ and that the NRMSE values for this relationship are relatively low. Therefore, leaf chlorophyll can be

said to have strong potential for use as a proxy for Vcmax$_{25}$. These findings are very promising in terms of the use of satellite remote sensing data for large-scale estimation of Vcmax$_{25.}$

Author Contributions

Conceptualization, Liangyun Liu; Formal analysis, Xiaojin Qian; Investigation, Xiaojin Qian, Holly Croft and Jingming Chen; Methodology, Xiaojin Qian; Writing – original draft, Xiaojin Qian; Writing – review & editing, Liangyun Liu and Holly Croft.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Table 1. Information about the samples.

| Plant species	Type	Number	Location	Measurement date
Cotton (*Gossypium spp*)	Crop	23	Baoding	July 2017
Wheat (*Triticum aestivum*)	Crop	84	Beijing	April to June 2018
Forsythia (*Forsythia suspensa*)	Shrub	5	Beijing	July 2017
Piemarker (*Abutilon theophrasti*)	Shrub	6	Beijing	July 2017
Honeysuckle (*Lonicera japonica*)	Shrub	16	Beijing	October 2017
Chinese redbud (*Cercis chinensis*)	Shrub	10	Beijing	October 2017
Red maple (*Acer rubrum*)	Tree	16	Ontario	May to October 2014
		14		May to October 2015
Bigtooth aspen (*Populus grandidentata*)	Tree	13	Ontario	June to September 2014
		10		June to October 2015
Trembling aspen (*Populus tremuloides*)	Tree	16	Ontario	May to October 2014
		13		May to October 2015
White ash (*Fraxinus americana*)	Tree	14	Ontario	June to October 2014
		12		May to October 2015
		13	Beijing	October 2017
Long bean (*Vigna unguiculata*)	vegetable	11	Sanya	November 2018
Radish (*Raphanus sativus*)	vegetable	3	Sanya	November 2018
Chinese cabbage (*Brassica pekinensis*)	vegetable	4	Sanya	November 2018

Table 2. Model statistics for linear regressions of log-transformed Jmax against Vcmax for the data collected in this study, from Walker et al. (2014), the TRY database and Wullschleger (1993).

	Model term	Coefficient	R^2	P-value
This study	Slope	0.91	0.79	<0.001
	Intercept	1.16		
Walker	Slope	0.89	0.87	<0.001
	Intercept	1.01		
TRY/Kattge	Slope	0.75	0.79	<0.001
	Intercept	1.67		
Wullschleger	Slope	0.84	0.87	<0.001
	Intercept	1.43		

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

Model	Combination	Formula	NRMSE	Bias
Model1	Equations (9) and (11)	Vcmax = 1.46 × Chl − 3.11	0.13	-4.56
Model2	Equations (9) and (12)	Vcmax = 1.11 × Chl + 10.89	0.14	-6.70
Model3	Equations (10) and (11)	Vcmax = 1.70 × Chl − 6.49	0.13	3.13
Model4	Equations (10) and (12)	Vcmax = 1.28 × Chl + 8.33	0.12	-1.42

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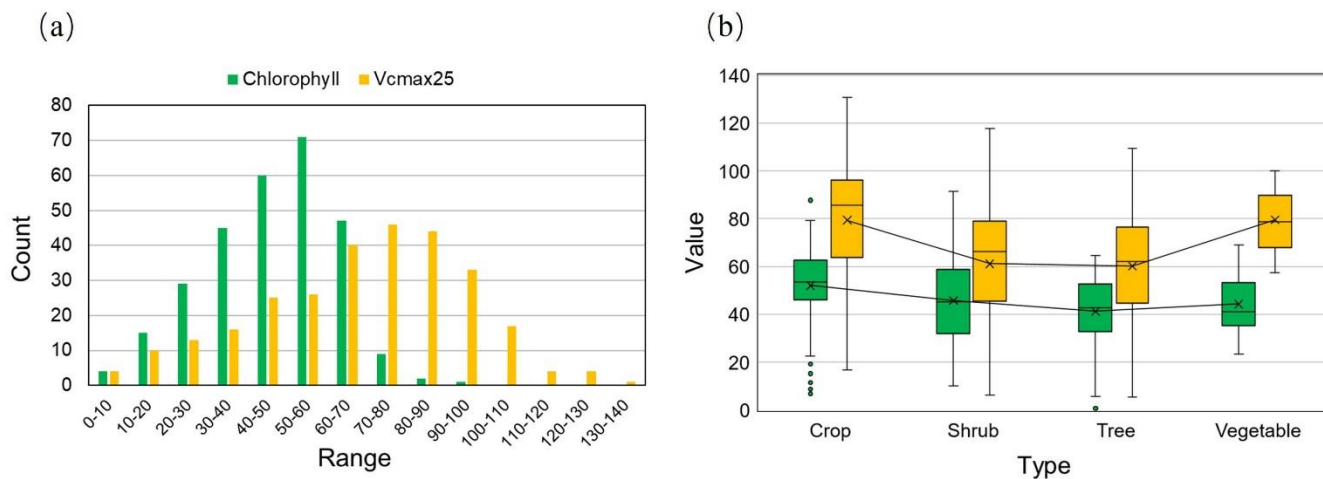
Figure 1. Distribution of chlorophyll content and Vcmax$_{25}$ values. In panel (a), the x-axis units are µg cm^{-2} for chlorophyll content and µmol m^{-2} s^{-1} for Vcmax$_{25}$. In panel (b), the y-axis units are the same as for the x-axis in panel (a). The horizontal line and cross within each box represent the median and mean, respectively; and the lower whisker extends to the minimum and the upper whisker to the maximum value, respectively. The green dot is an outlier datum point.
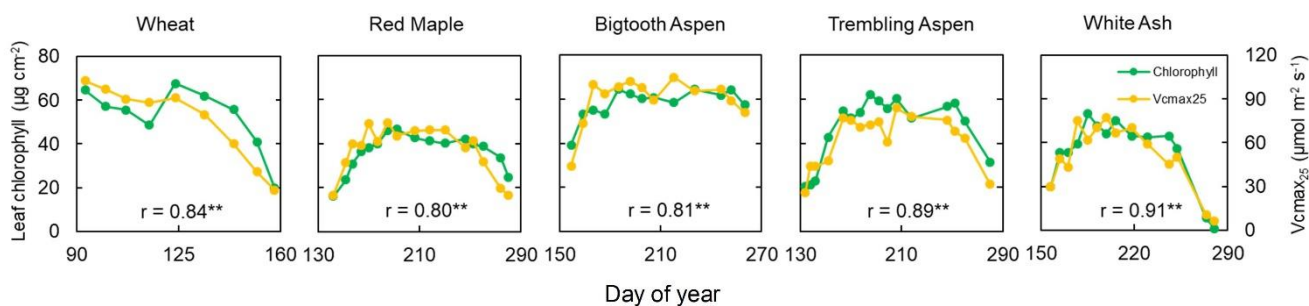

Figure 2. Variations in leaf Vcmax$_{25}$ and chlorophyll content during the growing seasons for wheat in 2018 and trees in 2014. The double asterisk represents a correlation that is significant at the 0.01 level (two-tailed test).
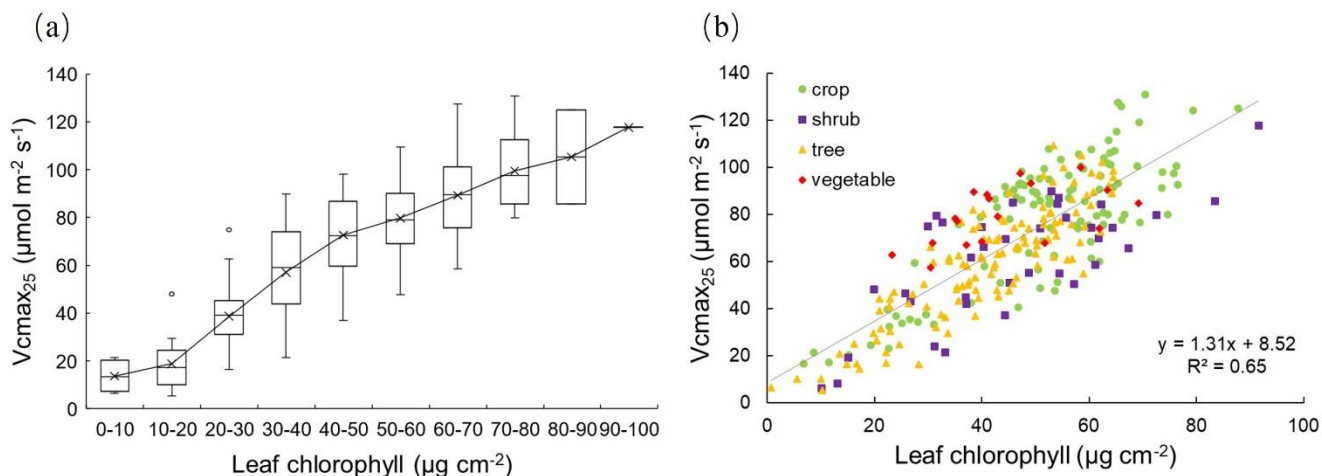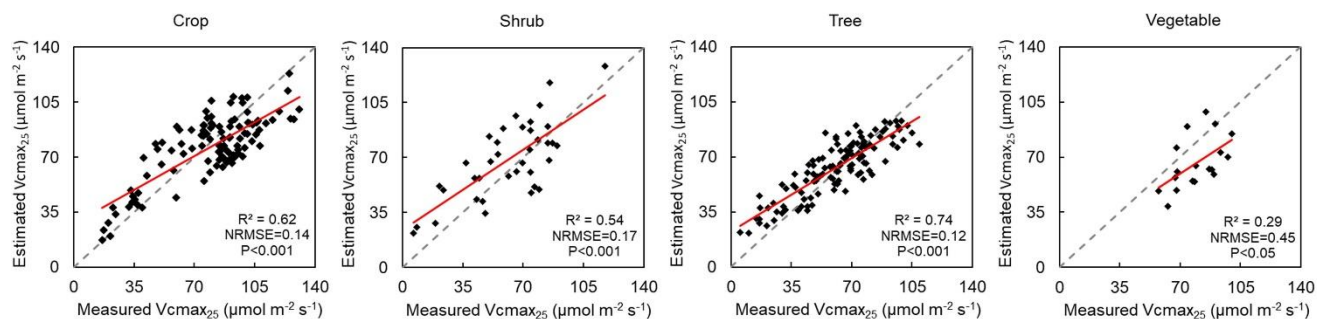
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(a)

(b)

Figure 3. Relationships between leaf Vcmax$_{25}$ and chlorophyll content across different C$_3$ plant types.

Crop

Shrub

Tree

Vegetable

425 **Figure 4. The correlations and normalized root mean square error (NRMSE) values between estimated and measured Vcmax$_{25}$ for crops, shrubs, trees and vegetables.**