

The triose phosphate utilization limitation of photosynthetic rate: out of global models but important for leaf models

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May 4, 2021

Opinion

Plant Cell & Environment

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Xiao et al. (2021) present a method for estimating the variability of estimated parameters of the Farquhar, von Caemmerer, Berry (FvCB) model of photosynthesis (Farquhar et al., 1980). This model has been very effective at predicting photosynthetic responses to CO₂, light, and temperature. The original model assumed one of two conditions: (1) rubisco is saturated with ribulose 1,5-bisphosphate (RuBP) and so responds to CO₂ with Michaelis-Menten kinetics (with a competitive inhibitor/ second substrate oxygen) (rubisco-limited) or (2) rubisco uses RuBP as fast as it is made (RuBP regeneration-limited). In that case, rubisco activity is determined by the rate of RuBP regeneration, typically as a result of being light-limited. But even though photosynthetic CO₂ assimilation (A) is light limited, it responds to increasing CO₂ because of suppression of photorespiration. Carboxylation plus oxygenation stays constant under RuBP regeneration limited conditions so if oxygenation goes down as CO₂ increases, carboxylation will go up. The model was expanded to include a third condition, where RuBP regeneration is limited by how fast phosphorylated intermediates, primarily triose phosphates, are converted to end products, thereby releasing phosphate (Sharkey, 1985). This is usually called “triose phosphate utilization (TPU) limitation.” Xiao et al. (2021) limited their analysis to rubisco-limited and RuBP-regeneration-limited fittings and said that TPU could also be included. We have tested how inclusion of TPU affects parameterization of the FvCB model.

The model is most often parameterized by measuring CO₂ assimilation as a function of CO₂ inside the air

spaces of the leaf (C_i), called an A/C_i curve. Rubisco-limited data points show a strong response to CO_2 while RuBP-regeneration-limited points show less response but still increase with increasing CO_2 . TPU-limited points are characterized by no response to CO_2 and sometimes an inhibition under increasing CO_2 . The condition is further diagnosed by a decline in photosynthetic electron transport caused by an increase in CO_2 or decrease in O_2 (measured by chlorophyll fluorescence analysis). The TPU limitation is rarely seen at physiological CO_2 partial pressure and temperature but is very frequently seen when CO_2 is marginally higher than what the plant experienced during growth, especially if the temperature during the measurement is marginally lower than the growth temperature. TPU conditions are also associated with oscillations in photosynthetic rate (Sharkey et al., 1986), complicating measurements of TPU-limited photosynthesis rates.

The parameters that can be estimated by the fitting models are the maximum rate of rubisco turnover (V_{cmax}) and the rate of electron transport (J) (since the analysis can be done at limiting light, this need not be J_{max}). Also estimated are respiration in the light (previously called day respiration) (R_L) and mesophyll conductance (g_m). If TPU is considered, this rate of triose phosphate use (TPU). We have used equations proposed by Busch et al. (2018) to include carbon flow out of photorespiration as glycine (a_G) or serine (a_S).

Some groups have concluded that TPU limitations are likely to be small and thus constitute an unnecessary complication for modeling photosynthesis at global scales (Kumarathunge et al., 2019; Rogers et al., 2021), and Xiao et al. (2021) also left TPU out of their recent analysis describing Bayesian methods for estimating parameters of the FvCB model and the uncertainties in those estimates. Given the observations of declining A and photosynthetic electron transport in their data we believe ignoring TPU can lead to errors. We have systematically explored the consequences of including or ignoring TPU when parameterizing the FvCB model when TPU is apparent in the data.

We began by re-analyzing the experimental data provided by Xiao et al. (2021). Four A/C_i curves measured with rice were provided. In three out of four cases, reverse sensitivity to CO_2 of A was observed and in all four cases, photochemical yield (measured by chlorophyll fluorescence analysis) declined at high CO_2 . These behaviors indicate that TPU was occurring. The authors specified in their methods section that they had to wait much longer for stability at the high CO_2 concentrations and the data at high CO_2 was noisy, also an indicator of TPU. We tested the effect of adding TPU to the analysis.

We converted the most recent version (2.9) of the fitting spreadsheet that has been provided by Plant Cell and Environment (Sharkey, 2016) to an R script with a user-friendly interface (Shiny app), see <https://github.com/poales/msuRACiFit>.

The script iteratively fits data sets to biochemical models using rubisco-limited, RuBP-regeneration-limited, or TPU-limited assumptions, then calculates which process is likely to be rate-limiting for each data point, thus eliminating the need to assign specific limiting process to each of the data points.

We then fitted the data supplied by Xiao et al. (2021), first without TPU and then with TPU (Figure 1). For all four curves supplied (only repetitions 2 and 3 are shown in Figure 1), including TPU in the fitting improved the fit to the data at high CO_2 and this was reflected in a reduction in the sum of the squared residuals (SSR) (data for repetitions 2 and 3 are given in Table 1). The reduction in SSRs was much greater than the increase in degrees of freedom introduced by including TPU as a fitting parameter.

When data points are treated as J -limited but are actually limited by another process such as TPU, then J is likely to be underestimated. The estimate of J was higher when TPU was included in the analysis (Table 1). Our fitting program could not estimate g_m when TPU-limited points were treated as being J -limited and hit the limit imposed during fitting of $100 \mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$. Because J -limited measurements hold the most information on mesophyll conductance, the estimate of mesophyll conductance is affected by fitting without TPU. When TPU is included it becomes clear how few data points are J -limited and since J -limited points have the most information about g_m it becomes clear why g_m can be difficult to measure when A/C_i curves are measured at saturating light. Using high but not saturating light can increase the amount of J -limited data when estimating g_m (Sharkey, 2019)(see box 1).

Three of the four A/C_i curves had noticeable discontinuity in the middle of the curves. We reasoned this was caused by the method used to make the measurements. It is common for researchers to report A/C_i curves assessed by measuring at 400 ppm CO₂ and then measuring at a series of declining CO₂ concentrations followed by a jump back to 400 ppm and measuring at a series of increasing CO₂ concentrations. We call this the split method and it requires that photosynthesis be identical before and after measuring photosynthesis at ambient CO₂, a requirement that often does not hold in our experience.

We examined the effect of the sequence of CO₂ concentrations measured during an A/C_i curve and conclude that these measurements should be made by monotonic increasing (or decreasing) CO₂ as opposed to starting at an ambient CO₂ concentration and going down in CO₂, jumping back to the middle and going up in CO₂ (we call this the split method).

We tested split versus monotonic methods with tobacco (Fig. 1 E and F). The curves did not show an obvious discontinuity but the SSR was higher for data generated by a split A/C_i curve than monotonic curve (Table 1) (These SSRs are comparable because the models used were the same and so the degrees of freedom did not differ.) Even when the curves do not show an obvious discontinuity in the middle when measured by the split method, results from “split” experiments tend to show stronger deviations from continuous fits to models, suggesting that hysteresis can strongly impact the interpretation. Moreover, the discontinuity comes at the section of the curve that has most information on mesophyll conductance and so significantly reduces confidence in mesophyll conductance values of such split curves.

We conclude that 1. it is important to include TPU when fitting A/C_i curves that show evidence for it; 2. A/C_i curves should be carried out monotonically. 3. Additional data may be needed depending on how the fittings are to be used, for example it may be necessary to measure curves at saturating and also substaurating light to get robust measures of all parameters. Because of the danger of over fitting, when possible, parameters should be fixed. For example, if there are independent measures of mesophyll conductance or light respiration, these can be supplied and then fixed during fitting. It must be accepted that some parameters can change within minutes and this biological source of variance should be considered. Very rapid, monotonic A/C_i curves are likely to be very helpful in assessing the physiology of photosynthesis just as a high speed shutter on a camera helps bring things into focus, especially when the subject is dynamic.

Reporting the parameters of the FvCB model can be helpful for global modeling, for detecting effects of the environment on photosynthesis, and changes in specific components of photosynthetic capacity. For large datasets fitting batches of curves using programs like R can be very helpful. What is presented expands on part of an earlier R Package (Duursma, 2015) but now includes TPU . The Shiny app allows users to test specific hypotheses and can be a convenient way to explore how changing conditions such as temperature and light affect predicted rates of photosynthesis.

Please see <https://github.com/poales/msuRACiFit> for how to access and use the R-script and Shiny app used for this work.

Funders: Division of Chemical Sciences, Geosciences and Biosciences, Office of Basic Energy Sciences of the United States Department of Energy (Grant DE-FG02-91ER20021).

			Rice rep 2	Rice rep 2	Rice rep 2	Rice rep 3	Rice rep 3
	Units	Units	Units	Without TPU	With TPU	With TPU	Without TPU
V_{cmax}	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	188	243	243	142
J	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	209	272	272	164
TPU	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	-	13.0	13.0	-
g_m	$\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$	100	8.3	8.3	100
R_L	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	5.5	7.6	7.6	0.77
a_T	unitless	unitless	unitless	-	0	0	-
a_Σ	unitless	unitless	unitless	-	0.53	0.53	-
SSR	$(\mu\text{mol m}^{-2} \text{s}^{-1})^2$	$(\mu\text{mol m}^{-2} \text{s}^{-1})^2$	$(\mu\text{mol m}^{-2} \text{s}^{-1})^2$	56.3	17.7	17.7	3.15

		Rice rep 2	Rice rep 3	Sequencing	
	Units	Without TPU	With TPU	Without TPU	With TPU
V_{cmax}	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	188	243	142	147
J	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	209	272	164	174
TPU	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	-	13.0	-	11.0
g_m	$\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$	100	8.3	100	30.1
R_L	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	5.5	7.6	0.77	0.79
a_G	unitless	-	0	-	0
a_S	unitless	-	0.53	-	0.37
SSR	$(\mu\text{mol m}^{-2} \text{ s}^{-1})^2$	56.3	17.7	3.15	1.07

		Rice rep 2	Rice rep 3	Sequencing	
	Units	Without TPU	With TPU	Without TPU	With TPU
V_{cmax}	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	188	243	142	147
J	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	209	272	164	174
TPU	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	-	13.0	-	11.0
g_m	$\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$	100	8.3	100	30.1
R_L	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	5.5	7.6	0.77	0.79
a_G	unitless	-	0	-	0
a_S	unitless	-	0.53	-	0.37
SSR	$(\mu\text{mol m}^{-2} \text{ s}^{-1})^2$	56.3	17.7	3.15	1.07

Table 1. Three comparisons of parameter values and sum of squared residuals (SSR) . Rice reps are the replicates from Xiao et al. (2021) showing the differences that occur when the triose phosphate utilization (TPU) limitation is considered and when it is not (fittings of the data in Figure 1 A-D). J will always be underestimated when TPU limited points are treated as being J -limited. Without the TPU limitation, our fitting program was unable to resolve g_m and so the arbitrary limit of 100 is shown. The last two columns show the effect of measuring the A/C_i relationship by the split method versus monotonically (low to high CO_2 in this case using *Nicotiana tabacum*, fittings of the curves shown in Figure 1 D and E). Although no discontinuity was apparent, the SSR was lower in the monotonic case. Multiple trials were made and SSR was always smaller when the curves measured monotonically rather than by the split method.

Figure 1. Fitting A/C_i curves. A and B fits to rice data (replication 2 of Xiao et al. 2021) without (A) or with (B) TPU, C and D are fits to replication 3. The effect of split A/C_i determination (E) or monotonic (increasing CO_2) (F) for *Nicotiana tabacum* . Red is the fitted shape for rubisco-limited condition, blue is for the RuBP regeneration-limited condition and gold is for the TPU-limited condition.

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